

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH
SUMMARY OF TOXICOLOGY DATA

ATRAZINE

Chemical Code # 000045, Tolerance # 00220, SB 950 # 008
August 11, 1986

Revised 7/10/87, 11/20/87, 5/23/88, 2/9/89, 5/26/89, 11/29/89, 3/22/90,
6/30/90, 2/25/92, 10/18/93, 12/7/93, and 4/20/94, 10/4/96, 6/1/98, 5/20/99, 10/15/99, 01/05/00
7/27/01 and 1/28/08

I. DATA GAP STATUS

Chronic rat:	No data gap, no adverse effect
Chronic dog:	No data gap, possible adverse effect
Oncogenicity rat:	No data gap, possible adverse effect
Oncogenicity mouse:	No data gap, no adverse effect
Reproduction rat:	No data gap, no adverse effect
Teratogenicity rat:	No data gap, no adverse effect
Teratogenicity rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome Effects:	No data gap, no adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	Not required at this time

In one-liners below, ** indicates acceptable study, whereas **bold faced** type for DPR Document/Record Numbers indicate a possible adverse effect.

All record numbers identified as relevant in data indexed by DPR as of 1/28/08 have been reviewed. These include record numbers through 180553 (in Document No. 220-0563), one higher record number in an older document (Record No. 230286 in Document No. 220-0104), plus any relevant record numbers over 900000.

Previous revisions by M. Silva, J. Gee, K. DiBiasio, and C. Aldous.

These pages contain summaries only. Individual worksheets may contain additional effects.

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NOTE: the Simazine Summary of Toxicology Data contains data relevant to atrazine.

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II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

COMBINED, RAT

NOTE: Studies using Sprague-Dawley female rats indicate that atrazine increases mammary tumor incidence, or reduces the latency to appearance of mammary tumors. Submitted data indicate that dose levels that elicit tumors also block the surge of luteinizing hormone (LH), which is necessary to stimulate ovulation. Vaginal cytology evaluations show that the prevalence of persistent estrus is enhanced after about 3 months of exposure to 400 ppm atrazine. Record No. 156970, p. 19, makes reference to a study, evidently by Tyrey *et al.*, (1996), which reported that the failure of high dose atrazine group females to produce the LH surge is **not** due to a lack of responsiveness of the pituitary gland to gonadotropin releasing hormone (GnRH). There is no report as yet that atrazine effects on GnRH levels have been studied. Studies to date evaluating possible atrazine estrogenic effects have **not** indicated remarkable effects on estrogen function. In particular, the 2-year dietary study in Sprague-Dawley rats, DPR Record No. 160554, found no mammary tumors in ovariectomized rats up to the highest atrazine dose of 400 ppm, administered throughout the study.

The most plausible explanation for the increased mammary tumors in Sprague-Dawley rats is that a perturbation in the control processes regulating reproductive hormones occurs at the level of the hypothalamus. Since the primary locus (loci) of the alteration is (are) not known, it cannot be determined what analogous changes would be expected in humans. Information provided thus far does not establish that a threshold phenomenon exists for atrazine effects on reproductive hormonal changes or possible consequences thereof, including tumor development.

There are so many studies that were performed to evaluate the basis of the mammary tumors in Sprague-Dawley females that the portion of this Summary on chronic toxicity and oncogenicity in rats has been subdivided into sections, with explanatory comments for the larger sections. Aldous, 6/1/98.

Guideline "Combined" Rat Study (and associated records)

**** 064-078 044294-044308** Mayhew, D., "Twenty-four Month Combined Chronic Oral Toxicity and Oncogenicity Study in Rats Utilizing Atrazine Technical." (American Biogenics Corp. [Toxigenics], 5/29/86.) Atrazine Technical (95.8%, lot FL-0821575); fed at 0, 10, 70, 500, 1000 ppm in the diet to Sprague-Dawley rats. NOEL for non-neoplastic effects = 70 ppm (diminished weight gain in both sexes at 500 and 1000 ppm, irritability in males and pale appearance and probable anemia in females at those dosages. **POSITIVE FOR ONCOGENICITY:** Female mammary tumors included dosage-related increases in adenocarcinomas and fibroadenomas: statistically significant for pairwise comparisons at 70 ppm and above for carcinomas and at 500 ppm and above for all mammary tumors. Testicular interstitial cell tumors were increased in the 1000 ppm males. Complete and **ACCEPTABLE** for primary review. Additional data or re-arrangement of existing data may be required for risk assessment group evaluation. C. Aldous, 7/16/86. **NOTE:** Results of CDFA and DPR re-evaluations of these data follow:

Re-examination #1: The above study (Doc. #s -064 through -078) found no significant adverse non-oncogenic effects below the LEL of 500 ppm. Weight gain decrements, reduced food consumption, and clinical signs were observed in a dose-related fashion at 500 and 1000 ppm, so that non-oncogenic NOEL was 70 ppm. Miscellaneous findings at 1000 ppm are given

in the report and do not indicate significant chronic risks. Incidence of fibroadenomas and more particularly of adenocarcinomas in female mammary gland indicate dose-related responses that extend to the 70 ppm level and will require risk assessment procedures. It is not clear whether the apparent increase in testicular interstitial cell adenomas are true treatment effects, as indicated in the review C. Aldous, July 86). There is no evidence for interstitial cell tumor induction at or below 500 ppm, indicating that such tumor risk is unlikely to be the basis for major oncogenic risk evaluation. Pituitary tumors were noted by investigators as increased in 1000 ppm females when differential mortality was considered, but such an increase was not apparent on inspection of incidence data. C. Aldous, 7/10/87.

Re-examination #2: [Purpose of this re-examination was to tabulate and discuss findings of potential concern]. There were no substantial differences from earlier conclusions: testicular interstitial cell tumors are possibly treatment-related in high dose males, as was prostate epithelial hyperplasia. An examination of evidences for a NOEL for anemia in females (including a re-examination of splenic extramedullary hematopoiesis and bone marrow myeloid hyperplasia) still supports a NOEL of 70 ppm. Several histopathology findings limited to 1000 ppm rats are included in this worksheet. Aldous, 12/2/93.

062 042305 (Experimental Pathology Labs) Pathology tables from interim report. Final study subsequently reviewed (See one-liner and comment on full study above: study in volumes 064-078, Rec. #s 044294-044308). J. Schreider, 4/18/86.

049 016884 (ToxiGenic, Inc., 1984) Twelve-month interim report. (See one-liner and comment on full study above: study in volumes 064-078, Rec. #s 044294-044308). J. Schreider, 5/09/85.

059 038012 Duplicate information of 049 016884.

220-407 138990 Hardisty, J., "Atrazine: Supplement to two-year chronic feeding/oncogenicity study in rats administered atrazine", EPL re-evaluation of female mammary tissue sections for the primary rat combined study in Record Nos. 044294-044308. Date of EPL report: Oct. 1, 1987. An individual EPL pathologist (G.I. Riley) performed this re-evaluation, whereas U.S. EPA currently requires a "pathology working group" evaluation to serve this purpose. Summary tables, recorded in the DPR review of this record, confirm essential conclusions noted in the existing 1-liner of Record No. 044294. In the 24-month rats, 500 and 1000 ppm groups had increases of benign tumor lesions such as "fibroadenoma, NOS" and in "proliferative fibroadenoma." Malignant tumor data indicated treatment effects at 70 ppm upwards, with the greatest contribution from two noninvasive tumor lesions: "tubulopapillary carcinomas" and "compact tubular carcinomas" (from pp. 10 to 16). Combined data for the 12- and 13-month intervals indicated a treatment response at the 1000 ppm level for the latter tumor forms at that time interval. Aldous, 10/4/96.

Mechanistic Studies in Sprague-Dawley Females

SUMMARY: The chronic (12/24 month) study (Record No. 151475) reaffirmed the mammary tumor development at 400 ppm in intact females, but found no mammary tumors at all to date in ovariectomized rats. This does not eliminate the possibility of an estrogenic effect of atrazine, since the vestigial mammary tissue in ovariectomized rats would likely have required substantial hormonal influence to develop sufficiently to express hormonally-mediated tumors. Vaginal smear data available thus far are consistent with earlier studies in demonstrating enhanced estrus

in females after about 3 months on treatment. Record No. 151472 provides the most definitive data available to date on effects of atrazine on the LH surge that precedes ovulation and completion of estrus, demonstrating an elimination of the surge at 400 ppm. At even higher atrazine levels, the magnitude of the prolactin peak was also reduced (LEL of 200 mg/kg/day and NOEL of 40 mg/kg/day for this effect: see the 4-week gavage exposure study in Record No. 146011, below). Very high dose levels of atrazine also led to reduced circulating estradiol levels (reduced estradiol at 200 to 300 mg/kg/day, LEL for this finding = 100 mg/kg/day: see Record No. 139110). It appears that the effect on the LH surge is the most important of the findings in this section. Aldous, 4/1/98.

220-511 160554 Morseth, S. L., “Chronic (12-24 month) study in rats with Atrazine Technical”, Covance Laboratories Inc., 4/15/98. Study No. 2386-108. Eighty intact Crl:CD7BR females per group were dosed with 0, 25, 50, 70, or 400 ppm Atrazine Technical (97.1%) in a 2-yr study. Twenty/group were designated for 1-yr interim sacrifice. Parallel groups of ovariectomized rats of the same size and dose levels were treated on the same schedule. Primary focus was histopathology of reproductive tissues and pituitary. Vaginal cytology smear samples were taken daily for 14 consecutive day periods every 4 weeks (see next paragraph). Apparent NOEL for non-neoplasia, excluding vaginal cytology data (not provided in this report) = 70 ppm (slightly reduced body weights at 400 ppm in ovariectomized and intact females). There was a decreased time-to-tumor for mammary carcinomas in 400 ppm intact rats. There were no mammary tumors in ovariectomized rats. DPR review compares these findings with those of other long-term studies using Sprague-Dawley females. Valid ancillary study. Aldous, 5/27/98.

220-539 170324 Thakur, A. K., “Addendum 1 to Study No. 2386: Chronic (12/24 month) study in rats with atrazine technical (EPA MRID No. 44544701).” These data are associated with DPR Document/Record No. 220-511 160554 (Covance Study No. 2386-108: see above). Study design and tumor data were previously reviewed under the latter record number. Eighty intact (i.e. not ovariectomized) Crl:CD7BR females per group were dosed with 0, 25, 50, 70, or 400 ppm Atrazine Technical (97.1%) in a 2-yr study. Of these rats, twenty/group were designated for 1-yr interim sacrifice. Vaginal cytology smear samples were taken for periods of 14 consecutive days, at intervals beginning every 4 weeks. The present study evaluated relationships between treatment levels, prevalence of estrus (as indicated by vaginal cytology), and carcinoma incidence. **General associations of mammary carcinomas and time in estrus:** Data revealed several important relationships, the strongest associations being between mammary carcinomas and vaginal cytological signs of estrus during weeks 17-26. This critical period marked a time of rapid change from normal estrous cycling to vaginal cytology signs of constant estrus. This was also the time of maximal difference in estrous state between control and 400 ppm rats. Control rats and dose levels of 25 through 70 ppm reached 50% of time in estrus at about week 22 of the study (about week 29 of age), compared to 400 ppm rats, which reached 50% of time in estrus at about study week 18. Some changes reflected this acceleration of age-related change in 400 ppm rats during study weeks 17-26 (the time period used for comparisons unless otherwise indicated): (1) 400 ppm rats averaged appreciably more time in estrus than controls and other groups, (2) rats that eventually bore mammary carcinomas averaged appreciably more time in estrus than rats in corresponding dose groups that did not have carcinomas, (3) 400 ppm rats with carcinomas had only marginally greater time in estrus than 400 ppm rats without carcinomas, (4) 400 ppm rats with carcinomas had only slightly greater time in estrus than rats in other treatment groups with carcinomas. There were no comparable associations of time in estrus and mammary fibroadenomas. **Associations of early-appearing mammary carcinomas (evident by week 53) and time in estrus.** Seventeen rats (including 8 of the 400 ppm rats) had early-appearing carcinomas. All 17 rats reached or

approached 100% of time in estrus by the time of the first palpation of a carcinoma. Two of these high dose rats had carcinomas palpable at weeks 14 and 18, respectively. These findings corresponded to the first periods in which these rats showed cytological signs of sustained estrus. Four of the other six high dose rats with carcinomas that were palpable within the first year had an increased percentage of days in estrus during weeks 17-26 compared to the study average: time in estrus was less than the study mean for the other two rats. The majority of rats in other groups that had carcinomas that were palpable within the first year had lower average time in estrus during this time period than the overall study average. Thus the limited numbers of rats with early-appearing carcinomas did not show a consistent association with increased time in estrus during weeks 17-26. Histopathology for this study and other studies had previously shown that the primary effect of chronic feeding of CD females with 400 ppm atrazine was increased incidence of early-onset mammary carcinomas. It is particularly interesting that there was no clear association between prolonged estrus (i.e., constant estrus over a period of several weeks), particularly during the time frame of weeks 17-26, and early-onset tumors in this study. Some period of constant estrus, most commonly of several weeks duration, almost always preceded the appearance of a carcinoma, and usually was present at the time that the tumors first became palpable. The need for some estrogen influence to allow mammary carcinoma development is consistent with the histopathology findings of Record No. 160554, which reported no mammary tumors in ovariectomized rats up to the highest atrazine dose of 400 ppm, administered throughout the 2-year study. Since prolonged estrus is not a necessary condition for early-onset mammary carcinomas in 400 ppm atrazine rats, it is possible that the general increase of time in estrus *per se* is not the sole or primary cause of the increase in early carcinomas in 400 ppm rats, but instead the altered vaginal cytology may be a visible manifestation of a more central toxicological response. Aldous, 5/20/99 (edited to add Record Number and Document Number on 10/14/99).

NOTE: Interim results of estrous cycling data from Study No. 2386-108 were mailed directly to Medical Toxicology Branch on 5/4/98. Data were presented as plots showing prevalence of persistent estrus or persistent diestrus, evaluated over time periods of week 0 to week 38, in each of the above treatment groups. Data suggest that 400 ppm rats entered persistent estrus by week 21, whereas controls and treated groups through 70 ppm did not reach this incidence of persistent estrus until about week 29. Given the large numbers of slides yet to be evaluated to complete the analyses, it will take some time before this report is completed. Aldous, 5/5/98.

220-458 151475 Interim report of Laboratory Study # CHV 2386-108. Aldous, 4/7/98. (See review of final report of histopathology data in Record No. 160554, above.)

220-457 151472 Morseth, S. L., "Evaluation of the luteinizing hormone (LH) surge in atrazine-exposed female Sprague-Dawley rats - 6-month report," Corning Hazleton Inc., 10/25/96. Laboratory Study # CHV 2386-111. Ninety Crl:CD7BR females per group were dosed in diet with 0, 25, 50, or 400 ppm Atrazine Technical, purity 97.1%, for 6 months. To assess estrous cycling, smear samples were taken daily for vaginal cytology for 14 consecutive days at 4 week intervals. Rats were ovariectomized 10 days before sacrifice. A capsule containing 4 mg estradiol per ml sesame oil was implanted 3 days before sacrifice. These measures ensured that sufficient estrogen was available to induce an LH surge regardless of treatment regimen. Blood was sampled after 6 months of treatment for prolactin and LH (study outcome parameters) and estradiol (to validate experimental technique) by RIA. Blood was sampled at intervals over a 12-hour period of the day during which surges of LH and prolactin are known to occur. Ten rats/group were bled 6 times over a 12-hour period on the day of sacrifice, providing sufficient blood for LH assays each time. The other 80 rats/group were sacrificed at intervals, to provide

sufficient blood for prolactin and estradiol assays as well as LH. [Reproductive tissues were preserved for histopathology: to be presented in a later report. An additional report will present pituitary hormone level data, from tissues collected in this study]. There were modest but statistically significant body weight and food consumption decrements at 400 ppm. Vaginal cytology data showed an impressive increase in prolonged or constant estrus at 400 ppm during the last 3 months of the study. At most sampling intervals for vaginal cytology, there was no indication of effects at lower dose levels. **More thorough evaluation is requested on vaginal cytology at weeks 21-22, at which time it appears that intermediate dose groups show prolonged or constant estrus.** The pulse of luteinizing hormone, which normally appears late in the daylight period in ovariectomized rats under estradiol treatment, was effectively blocked at 400 ppm. NOEL for body weight and food consumption decrements and for lack of the normal luteinizing hormone surge in estradiol-primed rats was 50 ppm (3.65 mg/kg/day). There was no definitive NOEL for estrous cycling alterations, although sustained effects were limited to 400 ppm rats. Aldous, 4/7/98.

220-429 146011 Morseth, S. L., "Evaluation of the luteinizing hormone (LH) surge in atrazine-exposed female Sprague-Dawley rats," Corning Hazleton Incorporated, Vienna, VA (CHV), 1/25/96. CHV Study No. 2386-111. Ninety Crl:CD7(SD)BR females/group were dosed daily by gavage (in 0.5% aq. CMC) for 28-31 days prior to ovariectomies, and continuing for 10 days afterwards, with 0, 2.5, 5, 40, or 200 mg/kg/day atrazine. During weeks 2-4 of treatment (prior to ovariectomy), rats were evaluated for estrous cycle stage by vaginal smear analysis. Seven days after ovariectomy, rats were implanted with silastic capsule designed to provide estradiol (E2) levels ≥ 12 pg/ml. On the tenth day after ovariectomy, blood samples were taken at intervals (20-25 samples/interval) for assays of E2 (to verify implantation), LH, and prolactin. Prior to ovariectomy, estrous cycling was disturbed, most remarkably by prolonged periods of diestrus at 40 and 200 mg/kg/day. Duration of estrus was also prolonged at 200 mg/kg/day. Ovariectomized rats provided with E2-releasing implants had remarkable decrements in LH peak levels at 40 and especially at 200 mg/kg/day. There was also a reduction in peak prolactin levels at 200 mg/kg/day, with a possible delay in timing of prolactin peak levels. Data are consistent with the hypothesis that the primary atrazine toxic event leads to delays of ovulation by disturbing the surges of releases of LH and prolactin. The author suggests that data support a "threshold," however spacing of dose levels and high response variability do not allow a definitive decision on that matter. This is a valid ancillary study. Aldous, 10/4/96.

220-424 146005 Protocol for CHV Study No. 2386-111, for which the gavage portion has been reviewed (DPR Record No. 146011, above). No worksheet is needed. Aldous, 9/5/96.

220-427 146009 Morseth, S. L. Method validation for 220-429 146011, above. No worksheet is necessary. Aldous, 8/14/96.

220-428 146010 Morseth, S. L. "Evaluation of the luteinizing hormone surge in female Sprague-Dawley rats - Method validation," Corning Hazleton Inc., 1/18/96. Laboratory No. CHV 2386-110. Relates to 220-429 146011, above. One dose of atrazine (300 mg/kg/day) was tested in repeat-bled rats only. There was an apparent reduction in the LH surge. Prolactin levels did not return to baseline in the course of the day: a possible indication that the stress associated with repeat bleeding may lead to invalid prolactin data. [Note that prolactin was not assayed in repeat-bled rats in the primary study (Record No. 146001)]. No worksheet is necessary. Aldous, 8/14/96.

220-409 139110 Morseth, S. L., "Fourteen-day repeated dose oral toxicity/hormone study in female albino rats," Hazleton Laboratories America, Inc., March 6, 1990. HLA Study No. 483-268. Groups of 15 female Crl:CD7BR rats were dosed for at least 2 wk with 100, 200, or 300 mg/kg/day of atrazine (97%) or diaminochlorotriazine (DACT: 97.4%). High doses had been originally 400 mg/kg/day, but were reduced to 300 mg/kg/day for both test compounds on day 4 due to excessive toxicity. Two groups of 15 each served as controls: one received only corn starch suspension vehicle, and the other (a positive control for prolactin secretion) received an ip dose of metoclopramide 20 min before sacrifice. Rats were sacrificed at the time of first determination of diestrous stage after at least 14 daily treatments, cycle stage being determined by vaginal cytology. Serum collected at sacrifice was assayed for prolactin, LH, FSH, progesterone, and estradiol (E2). Higher dose levels were clearly toxic: the majority of atrazine rats at 200 to 300 mg/kg/day as well as DACT rats at 100 to 400 mg/kg/day had clinical signs of "thin" or "few or no feces." All DACT groups and 200 to 300 mg/kg/day atrazine rats had dose-related b.w. decrements. All atrazine and DACT groups had marked and dose-related decrements in thymus weights as the primary organ weight finding. Coincident with the above signs of general toxicity, there were possible hormone level changes, particularly decreases of LH, progesterone, and E2 in 200 to 300 mg/kg/day DACT rats. In general, the high variabilities evident in hormone levels, coupled with high general toxicity in groups appearing to manifest hormone level changes, makes this study of very limited value for assessing intrinsic effects of test articles on hormone controls. Newer, more refined studies are more valuable than this study. Aldous, 10/4/96.

220-425 146007 Pettersen, J. C. and J. C. Turnier, "1-year chronic toxicity study with atrazine technical in rats," Ciba-Geigy Corporation, Farmington, CT, Dec. 8, 1995. Lab Study # F-00171. The present study focused on histopathology of reproductive tissues (only mammary and pituitary gland tissues were systematically examined for this report) in female Crl:CD7(SD)BR rats dosed for up to 1 yr with 0, 15, 30, 50, 70, or 400 ppm atrazine. There were 55 rats per group, with interim sacrifices of 10/group at 3, 6, and 9 months, and 25/group maintained for 1 yr terminal sacrifice. Estrous cycle stage was evaluated by vaginal cytology at each interim time, and blood was sampled at these times for reproductive hormone assays: these data are to be reported separately. NOEL [excluding vaginal smear data] = 50 ppm (very slight b.w. decrement at 70 ppm). There was a significant decrement in body weight and modest reduction in food consumption at 400 ppm. There was a significant increase in mammary tumor bearing rats at 400 ppm. Most tumors were adenocarcinomas. There were no other important histopathology effects, and lower dose groups were unaffected. Investigators' tumor onset time analyses found a significant trend toward reduced time to tumor for the categories of "benign" mammary tumors and for "all mammary tumors." Removing the high dose group from analyses eliminated significance in all cases. This is a valid ancillary study, confirming the capacity of atrazine to elicit mammary tumors within a 1-yr time frame. Since there was no testing in the range just below 400 ppm, there is no information on dose-response at dose levels approaching the MTD. Aldous, 10/3/96.

Note: Initial examinations of the preliminary vaginal smear data appeared to show some increases cases of "≥ 3 days in estrus" at dose levels as low as 15 ppm (Aldous comments on 4/6/98 and 6/1/98). Subsequently more vaginal smear data have been submitted, and no coherent pattern has been evident below 400 ppm atrazine. Aldous, 1/5/00.

220-377 137872 Preliminary report of Lab Study No. F-00171 (Record No. 146007, above).

Mechanistic Studies in Male Rats (any strain)

Stoker, T. E., S. C. Laws, D. L. Guidici, and R. L. Cooper, "The effect of atrazine on puberty in male Wistar rats: An evaluation in the protocol for the assessment of pubertal development and thyroid function," *Toxicological Sciences* **58**, 50-59 (2000). National Health and Environmental Effects Research Laboratory, U. S. EPA, Research Triangle Park, NC. Male Wistar rats were dosed with atrazine by gavage at levels of 0, 12.5, 25, 50, 100, 150, or 200 mg/kg/day from PND day 23 to PND 53 or 54 (except for some which were killed earlier for LH receptor evaluations). Pair-fed rats' diets were limited to match the food consumption of the 200 mg/kg/day group. Measurements included body weights, organ weights (ventral prostate, seminal vesicle with coagulating gland), time to preputial separation, serum estradiol and estrone levels, intra-testicular testosterone at PND 45 and PND 53, testicular LH receptor density on PND 45 and PND 53, and PND 53 serum levels of TSH, T3, T4, LH, prolactin, plus pituitary levels of the latter two hormones. Body weights were reduced in dose-related fashion at 100 mg/kg/day upwards. Controls weighed 281 g at PND 53, compared to 200 mg/kg/day rat mean weight of 233 g and pair-fed group weight of 239 g. The most sensitive finding justified by the data in this report is reduction of ventral prostate weights, with a NOEL of 25 mg/kg/day. Lateral prostate and testicular weights were comparable to controls. Next in order of sensitivity is elevated serum estradiol, with a NOEL of 50 mg/kg/day. At these NOEL's there was no alteration of body weights. Investigators discussed a proposed "NOAEL" of 12.5 mg/kg/day for preputial separation in this paper, however this NOAEL is not supported by the data (which are tabulated in the DPR review, and which do not show dose-response over a wide dosage range). Useful supplemental data. Aldous, 7/25/01.

Stoker, T. E., C. L. Robinette, and R. L. Cooper, "Maternal exposure of atrazine during lactation suppresses suckling-induced prolactin release and results in prostatitis in the adult offspring," *Toxicological Sciences* **52**, 68-79 (1999). National Health and Environmental Effects Research Laboratory, U. S. EPA, Research Triangle Park, NC. Most studies involved treatment of Wistar dams during lactation days 1-4 with 0, 6.25, 12.5, 25, or 50 mg/treatment/day (2 treatments/day, by gavage) of atrazine. The normal suckling-induced prolactin secretion into the blood, which occurs in lactating rats, was studied in dams fitted with indwelling cardiac catheters on day 3. Prolactin secretion was completely inhibited at 50 mg/kg/dose atrazine. Controls and 6.25 mg/kg/dose atrazine rats had normal suckling-induced increases of prolactin. About half of the dams in intermediate groups had inhibition of prolactin secretion. Bromocriptine, a dopamine agonist known to inhibit prolactin release, when administered sc on the same schedule as atrazine, completely eliminated prolactin release at 0.104 mg/kg/dose and above, but not at 0.052 mg/kg/dose. To assess prostate inflammation in male offspring which were exposed via milk of dams which were treated twice daily with atrazine as described above during lactation days 1-4, the young adult offspring were sacrificed at day 90 or 120 for assessment of inflammation of lateral prostates (using myeloperoxidase activity as an index of inflammation, and confirming by histopathology). There was no statistical change at 90 days, however at 120 days, atrazine doses of 12.5 mg/kg/dose significantly increased prostate inflammation incidence. Bromocriptine at dose levels of 0.104 mg/kg/dose and above also significantly increased inflammation incidence. The importance of prolactin in milk for normal neurological development in neonates was supported by a subsequent test in which dams received ovine prolactin (sc, 0.3 mg/kg) on the same schedule as atrazine treatments with 25 or 50 mg/kg/dose atrazine. The combination of prolactin and atrazine eliminated the prostate inflammation which was present with atrazine alone. To assess critical periods of exposure, additional dams were treated twice daily with 25-50 mg/kg/dose on lactation days 6-9 or 11-14, and inflammation in prostates of offspring was evaluated on day 120. Treatment in the latter periods had no significant influence on prostate inflammation. It had previously been shown that in rats, milk-derived prolactin during the first

days of lactation is critical for development of the tuberoinfundibular dopaminergic system. This system in adult rats, as in humans, provides an inhibitory control on pituitary release of prolactin. Abnormal development of this system (as by neonatal exposure to dopamine agonists such as bromocriptine) can lead to inadequately controlled prolactin secretion and an associated lateral prostate inflammation as rats mature. Investigators were uncertain whether a comparable effect occurs in humans, in which the tuberoinfundibular dopaminergic system is largely organized before birth. Although there were several results of this study which implicated altered early-neonatal prolactin levels as the cause of subsequent lateral prostate inflammation, assays of serum or pituitary prolactin in male offspring at 120 days postpartum did not demonstrate an alteration of prolactin levels with treatment with atrazine or with bromocriptine. This suggests that the prostate inflammation is not dependent on altered prolactin levels in the maturing adult. NOEL = 6.25 mg/kg/dose (= 12.5 mg/kg/day for a 3 or 4-day exposure), based on reduced suckling-induced prolactin secretion in dams, and on lateral prostate inflammation in male offspring at day 120. Useful supplemental data. Aldous, 7/23/01.

Cooper, R. L., T. E. Stoker, L. Tyrey, J. M. Goldman, and W. K. McElroy, "Atrazine disrupts the hypothalamic control of pituitary-ovarian function," *Toxicological Sciences* **53**, 297-307 (2000). National Health and Environmental Effects Research Laboratory, U. S. EPA, Research Triangle Park, NC. Most mechanistic studies involved Long-Evans (LE) and Sprague-Dawley (SD) females dosed 1, 3, or 21 days at 0, 50, 100, 200, or 300 mg/kg/day. [1]. **Hormone secretion effects (especially LH and prolactin):** One series of experiments evaluated atrazine effects on levels of five hormones (LH, FSH, prolactin, TSH, and estradiol) in serum and anterior pituitary gland samples. These studies involved ovariectomized LE and SD rats implanted with estrogen-filled silastic capsules 3 days before sacrifice. Rats were treated at above doses for 1 or 3 days, respectively, then were terminated at 0, 1, 3, or 6 hr after final treatment, for the above hormone assays. Only LH and prolactin gave treatment responses, therefore only the latter two hormones were assayed in an analogous experiment using daily dose levels of 0, 75, 150, or 300 mg/kg/day for a 21-day exposure. **Serum hormone surges:** After a single dose, only the 300 mg/kg group of LE rats responded: these rats had marked inhibition of LH and prolactin surges evidenced by low LH and prolactin levels in serum samples at all post-treatment sampling times. After the 3-day treatment, LE rats had delayed response times for LH and prolactin surges at 50 mg/kg/day (spikes did not appear until about 6 hours, compared to the control peaks at about 3 hours), and secreted amounts for both hormones were markedly reduced at all time periods examined at all higher dose levels. SD rats after the 3-day treatment had significantly reduced prolactin levels at hrs 1-6 at 300 mg/kg/day only, but no alterations at lower doses with prolactin and none at all with LH. The 21-day exposure groups showed significant reductions in surge levels of LH and prolactin in both rat strains at the expected diurnal peak surge time at 150 and 300 mg/kg/day. In addition, LH was significantly reduced at 75 mg/kg/day in LE rats. **Pituitary hormone levels:** Pituitary samples were assayed for these two hormones in the 21-day groups. There were no treatment responses for pituitary LH levels, however pituitary prolactin levels were elevated at 75 to 300 mg/kg/day in both strains after 21 days. Prolactin levels were also measured in pituitaries of LE and SD rats following 3-day exposure. In that case, pituitary prolactin levels were consistently reduced at 6 hr at all dose levels tested (50 to 300 mg/kg/day) in LE rats, whereas there was no treatment effect in SD rats. [2] **Ovulation and estrous cycling effects:** Numbers of oocytes shed were counted in LE females by flushing the oviducts of rats which were administered up to 300 mg/kg atrazine on the day of vaginal proestrus, then sacrificed on the day of the following estrus. There were no treatment effects at any dose tested. When normally cycling, similarly treated LE rats were monitored for estrous cycling by daily vaginal smears for 3 weeks after a single proestrus dose of atrazine, 7/9 of the 300 mg/kg females became pseudopregnant, whereas none of the lower dose

groups (75 or 150 mg/kg) had abnormal cycling. **[3] Ectopic pituitary studies:** To identify whether atrazine affects pituitary function by direct interaction with the lactotrophs, LE rats were hypophysectomized and autotransplants of pituitary tissue were placed within the kidney capsule. After 4 wks recovery, rats were treated once with 300 mg/kg atrazine, and blood samples were taken hourly (tail vein) for 6 hr for prolactin assays. Prolactin levels were unchanged under these circumstances, however there were no associated controls for method validation. **[4] Exogenous gonadotropin-releasing hormone (GnRH) effects:** To evaluate effects of GnRH, ovariectomized LE rats were implanted with estrogen-filled silastic capsules, then dosed with 300 mg/kg atrazine for 3 days. Just after the third dose, rats were fitted with intra-cardiac catheters, and shortly thereafter given 50 ng/kg GnRH via catheter. Blood was sampled at time 0 and over the next 60 minutes to assay for LH. Atrazine alone blocked LH secretion, but the combination of atrazine and GnRH led to peak LH concentrations similar to control rats noted above. This suggests that the lack of an LH surge results from lack of stimulation of pituitary endocrine cells by hypothalamic GnRH. **[5] Responsiveness of pituitary tissues in vitro to hypothalamic hormone stimulation:** An *in vitro* perfusion study evaluated possible direct action of atrazine on pituitary LH secretion. Portions of anterior pituitary were taken from ovariectomized, estrogen-primed SD rats, and tissues were maintained in perfusion chambers and exposed to 0 or 100 μ M atrazine. LH or prolactin levels in medium were measured for over 2 hr as hormone levels diminished. Subsequently, medium was treated twice for 30-min intervals (spaced 1 hr apart) with GnRH (85 nM) plus thyroid releasing hormone (TRH, 100 nM). There was no difference between atrazine and control treatment responses of LH or prolactin secretion peaks upon challenge by releasing hormones. In a parallel *in vivo* exposure study, in which females were treated for 3 days by gavage with 0, 100, or 200 mg/kg/day atrazine prior to sacrifice and collection of pituitary gland samples for a comparable *in vitro* perfusion study, there were similarly no decreases in LH or prolactin secretion peaks upon challenge by releasing hormones. Instead, investigators noted an initial increase in prolactin release in atrazine-treated rats, consistent with elevated pituitary prolactin levels from atrazine treatment reported in part [1], above. This result also supports the concept that atrazine does not directly affect the pituitary, but interacts via interfering with secretion of releasing hormones from the hypothalamus. Useful supplemental data. Aldous, 7/27/01.

Studies Contrasting Hormone Level and Reproductive Tissue Histopathology of Sprague-Dawley vs. F344 Female Rats

SUMMARY: Increased mammary tumor incidence and/or decreased time to mammary tumor developments have been consistently shown in Sprague-Dawley female rats, but not in F-344 rats. The following studies have focused on histopathology of reproductive organs in these strains, as well as vaginal cytology (to determine patterns of estrous cycling), and blood hormone levels. The following studies show that a pattern of prolonged or constant estrus, which is a normal feature of aging Sprague-Dawley females, is brought on prematurely in Sprague-Dawley females exposed to 400 ppm atrazine, but not at levels as low as 70 ppm. Record No. 137871 indicated increased estradiol levels in 400 ppm Sprague-Dawley females at 9 months into the study. Prolactin levels were reportedly elevated in that group at 9 months, however it should be noted that prolactin levels were not remarkably affected in long-term studies of ovariectomized rats implanted with estradiol capsules, except for a reduction of peak levels of prolactin at very high atrazine dose levels (see section on “**Recent Studies Relating the Surge of LH. . .**,” above). Record No. 137871 reported a dose-related increase of numbers of antral follicles at 70 to 400 ppm in Sprague-Dawley rats but not F-344 rats at 9 months; a finding consistent with ovaries not receiving hormonal signals to ovulate and move beyond estrus stage. The influences of reproductive hormones that appear to be eliciting the increased numbers or premature

appearance of mammary tumors in Sprague-Dawley rats were considered by investigators not to be relevant to human physiological patterns (Record No. 128816). Aldous, 4/1/98.

220-167 112325 “Determination of Hormone Levels in Sprague-Dawley Rats Treated with Atrazine” (A. K. Thakur, Hazleton Washington, Inc., Study No. 483-278, 10/17/91) Atrazine technical, 97%, was fed in the diet at 0, 70 or 400 ppm to 70 female Sprague-Dawley rats per group. Ten/group were sacrificed at 1, 3, 9, 12, 15, 18 months and survivors at 24 months. Selected tissues were examined for histopathology: pituitary, mammary glands, uteri, and ovaries. The stage of estrus was determined prior to sacrifice. Samples of blood were taken but the data are not included in this report. The time to the appearance of tumors (mammary gland and pituitary) during the first year was shortened at 400 ppm. The overall incidence, however, over the two years was similar to controls and not statistically significant. Body weight gain was also lower at 400 ppm. Chronic NOEL = 70 ppm. The study confirms a **possible adverse effect for tumor induction**. Supplementary to the combined study in the rat. Gee, 2/24/92.

220-186 123799 Eldridge, J. C., Wetzel, L.T., Tisdell, M. O., and Luempert, L.G., “Determination of hormone levels in Sprague-Dawley rats treated with Atrazine Technical.” Hormonal studies were performed at Bowman Gray School of Medicine, Winston-Salem, NC on samples from a study conducted at Hazleton Laboratories America, Inc. Date of hormonal studies report: April 8, 1993. (The histopathology aspect of the study using these same animals is reported as DPR Record No. 112325, HWA Study No. 483-278). Atrazine technical, 97%, was fed in the diet at 0, 70 or 400 ppm to 70 female Sprague-Dawley rats per group. Ten/group were sacrificed at 1, 3, 9, 12, 15, 18 months and survivors were sacrificed at 24 months. Prior to sacrifice, rats were examined daily by vaginal smears to provide a “10-day cytology index.” Blood collected at sacrifice was assayed for hormones, including estradiol and progesterone. Based on vaginal cytology, 400 ppm females had an increased percentage of days in estrus over the first 18 months. Estradiol levels were elevated over other groups at months 3 and 9 (statistically significant only at 3 months). Data had substantial variability, but were generally consistent with the hypothesis that elevated estrogenic stimulation in early adult life of Sprague-Dawley rats influenced the elevated incidence of mammary tumors (or earlier onset of such tumors). Useful ancillary data. Aldous, 10/18/93.

220-220 128813 (an earlier version of Record No. 123799, above: no review)

220-168 (3 parts) 112326 “Determination of Hormone Levels in Fischer-344 Rats Treated with Atrazine Technical.” (A. K. Thakur, Hazleton Washington, No. 483-279, 11/8/91.) Atrazine technical, 97%, was fed in the diet at 0, 10, 70, 200, or 400 ppm to 70 Fischer-344 female rats per group. Ten per group were sacrificed at 1, 3, 9, 12, 15 and 18 months and surviving animals at 24 months. Selected tissues were examined microscopically - mammary glands, pituitary gland, ovaries, and uteri of all animals. There was weight gain depression, especially early in the study, at 200 and 400 ppm. No other effects were reported. The incidences of mammary and pituitary tumors were comparable across groups and no evidence of an effect on time-to-tumor was noted. Supplementary data. Gee, 2/24/92

220-187 123801 Eldridge, J. C., Wetzel, L.T., Tisdell, M. O., and Luempert, L.G., “Determination of hormone levels in Fischer-344 rats treated with Atrazine Technical” [Study was done in parallel with Record No. 123799, which employed Sprague-Dawley females]. Hormonal studies were performed at Bowman Gray School of Medicine, Winston-Salem, NC on samples from a study conducted at Hazleton Laboratories America, Inc. Date of hormonal studies report: April 8, 1993. (The histopathology aspect of the study using these same animals

is reported as DPR Record No. 112326, HWA Study # 483-279). Atrazine technical, 97%, was fed in the diet at 0, 10, 70, 200, or 400 ppm to 70 female Fischer-344 rats per group. Ten/group were sacrificed at 1, 3, 9, 12, 15, 18 months and survivors were sacrificed at 24 months. Prior to sacrifice, rats were examined daily by vaginal smears to provide a "10-day cytology index." Blood collected at sacrifice was assayed for hormones, including estradiol and progesterone. The present study did not identify treatment-related changes in vaginal cytology to indicate changes in "percent of time in estrus," nor were there treatment-related changes in serum hormone levels nor deviations from any other of the normal estrous processes over time in these rats. Useful ancillary data, Aldous, 10/18/93. [Record # 220-221:145382 is an earlier version of this report, for which there is no separate DPR review].

220-376 137871 McConnell, R.F., "A histomorphologic reevaluation of the ovaries, uterus, vagina, mammary gland, and pituitary gland from Sprague-Dawley and Fischer-344 female rats treated with atrazine," [re-examination of slides from Hazleton Washington, Inc. Study No. 483-278 for Sprague-Dawley rats, and No. 483-279 for F344 rats (original reports were reviewed under DPR Record Nos. 112325, 112326, 123799, and 123801)]. Date of present document is 3/10/95. The re-examination was conducted (1) to determine whether the animals in these studies were indeed sacrificed at proestrus, as intended (assessed by vaginal cytology and by histomorphology of reproductive tissues), and (2) to re-examine several estrogen-sensitive tissues in these two strains of rats in order to ascertain why treated Sprague-Dawley females had increased mammary tumor incidence. Results: most F344 rats were in proestrus, as desired, during the pivotal age span of 3 to 12 months on study, whereas most Sprague-Dawley rats had histomorphological features of both proestrus and "continuous estrus" during months 9-12. There was no clear atrazine effect on cycle stage at any sacrifice time in either strain. The major hormone level changes were limited to Sprague-Dawley rats at 9 months, when there was a modest increase in estradiol (E₂) levels, and a more remarkable increase in prolactin levels at 400 ppm. Mammary gland histomorphology changes, commonly associated with aging rats, appeared prematurely (most noticeably at 9 months) in 400 ppm Sprague-Dawley rats. These changes included enhanced degrees of age-associated changes such as acinar/lobular development, secretory activity, and duct ectasia with galactocele formation. The primary ovarian effect was a marked increase of numbers of antral follicles at 400 ppm and slight increase at 70 ppm in Sprague-Dawley rats at 9 months. The author did not observe vaginal epithelium changes due to atrazine that would be typical of potent exogenous estrogens (there were no positive controls for comparison). Investigators concluded that atrazine elicits histomorphologic changes in Sprague-Dawley rats by interference with ovarian function, perhaps through alteration of LH secretion (which was not a measured parameter in this study). Impairment of the LH surge necessary for ovulation could lead to earlier onset of mammary tumors through mediation of endogenous estrogens and prolactin in Sprague-Dawley females. Reproductive senescence in F344 rats follows a different pattern, and no such atrazine effect is seen. An EPA critique of this study and the Ciba response are contained in 220-375 137870, below. Aldous, 10/4/96.

220-188 123802 Thakur, A. K., "Strain-dependent responses to long-term atrazine feeding in female Sprague-Dawley and Fischer-344 rats: Statistical analyses of mammary and pituitary tumors, body weight gain, and survival" [discussion of several studies: no new data], April 22, 1993. This short discussion refers to the 1986 rat combined study (American Biogenics Corporation, DPR Record No. 044294), and more particularly to four separate studies, two employing Sprague-Dawley rats, and two employing F-344 rats. As of the time of this submission, DPR had received only two of the four reports (one per rat strain, namely Record Nos. 112325 and 112326, with corresponding HWA Study Nos. 483-278 and 483-279,

respectively). The remaining studies were subsequently received under DPR Record Nos. 128811 and 129145 (see DPR reviews of the latter studies, performed in 1994). The conclusions of this submission were that (1) there was an earlier onset of mammary tumors in 400 ppm S-D females but not in 70 ppm S-D females, (2) neither time of onset nor total incidence of tumors was affected at any dose tested in F-344 rats, (3) mortality was elevated in S-D 400 ppm females, suggesting that the MTD had been exceeded, however mortality was unaffected in 400 ppm F-344 females, and (4) body weight gain decrements in both strains suggested that 400 ppm had achieved the MTD. Aldous, 10/13/93 (amended by Aldous on 4/12/94).

NOTE: See a more recent evaluation of these data by Eldridge *et al.*, below (Record No. 128816).

061 039984 (Hazleton Labs, 1961) J. Schreider, 4/18/86. Summary. See EPA one-liner. No adverse effect reported.

EPA one-liner: "Too few animals to determine organ weight changes at sacrifice to set NOEL. Poor viability-infections. Core grade: Supplementary. (Two-year feeding; Hazleton Labs, 1961; MRID # 00059211)."

053 016905 Exact duplicate of 061 039984.

220-014 942528 Histopathology supplement to 039984, above.

220-219 128811 "Oncogenicity Study in Sprague-Dawley Rats with Atrazine Technical," (A. K. Thakur, Hazleton Washington, Inc., HWA Study No. 483-275, 1/27/92). Atrazine technical, purity 97%, was admixed with the feed at concentrations of 0, 70 or 400 ppm and fed to 60 female Sprague-Dawley rats/group for 104 weeks. Study was supplementary to earlier studies, and focused on tissues under female hormonal influence: primary microscopic examinations were on uterus, ovary, pituitary, and mammary gland. NOEL = 70 ppm (increased mortality; reduced body weight). A decrease in time of onset of mammary tumors was noted at 400 ppm, although total rats with mammary tumor neoplasia was not affected. This scientifically valid ancillary study provides additional useful information about the influence of atrazine on mammary tumor development, but does not affect the designation of the test article as having a "possible adverse effect." Kishiyama and Aldous, 4/20/94. **NOTE:** Data were re-examined to evaluate incidence of mammary tumors first palpated within year 1 of study. Incidences of fibroadenomas arising during first year were 2, 1, and 4 for control, 70 ppm and 400 ppm groups, respectively. Respective mammary carcinoma incidences arising in the first year were 0, 1, and 6. Thus there was a treatment-related earlier onset for mammary carcinomas and for combined mammary tumor incidence at 400 ppm only. The effect on mammary carcinoma incidence is probably the most important outcome of the study. Aldous, 5/29/98.

****220-291 129145** Thakur, A. K., "Oncogenicity study in Fischer-344 rats with Atrazine Technical," Hazleton Washington, Inc., HWA Study # 483-277, 2/18/92. Atrazine, 97%, was fed in diets of 60 rats/sex/group at dose levels of 0, 10, 70, 200, and 400 ppm for 2 years. Histopathology was evaluated on the standard battery of tissues. NOEL = 70 ppm (modest body weight decrements in both sexes). Study is **acceptable**, with **no adverse effects**. Aldous, 4/11/94.

220-222 128816 Eldridge, J. C., Wetzel, L.T., and Tisdell, M. O., "The effects of atrazine on endocrine parameters in the female Sprague-Dawley rat: A threshold-mediated and strain-specific response for mammary tumor development," 9/22/93. This submission is an evaluation of the relationship of mammary tumors in Sprague-Dawley and F-344 rats to atrazine

exposure, and influences of atrazine on hormonal status and hormonally-mediated events that eventually result in mammary tumors. Primary studies cited were DPR Record Nos. 112325, 112326, 128811, and 129145. Findings of cited records have been reviewed previously by DPR. Investigator's conclusions are that the earlier onset of mammary tumors in Sprague-Dawley rats was related to changes in estrogen function, and that the changes have a threshold (effects at 400 ppm but not at 70 ppm). Their evaluation further concluded that this mechanism would not apply to humans, since the processes of neuroendocrine changes with age in the two species are quite different. This reviewer agrees that it is probable that humans would also have a hormonal response to atrazine, but the mechanism of that response is not known, and the presence of possible thresholds and the associated dose levels cannot be determined at this time. Aldous, 4/20/94.

220-368 137861 Eldridge *et al.*, "Short-term effects of chlorotriazines on estrus in female Sprague-Dawley and Fischer 344 rats," *J. Toxicol. Environ. Health.*, 43:155-167 (1994). (Conducted at Bowman-Gray School of Medicine, Winston-Salem, NC). Virgin rats (10-13 wk old) of Sprague-Dawley and F344 strains were dosed by gavage with 0, 100, or 300 mg/kg/day atrazine or simazine for 14 to 23 days (dosing was stopped and rats were sacrificed when vaginal smear data first indicated proestrus stage, after a minimum of 14 days of treatment). Simazine effects were generally small or absent compared to atrazine effects, so that this review addresses strictly atrazine effects. Body weights at sacrifice for Sprague-Dawley rats were reduced 13% and 27% compared to controls, suggesting dose levels exceeding "MTD" criteria. Both strains showed dose-related absolute and relative decreases in ovarian and uterine weights, and absolute and relative increases in adrenal weights. Plasma hormone level changes included a dose-related drop in estradiol levels (significant only in Sprague-Dawley rats), and a significant elevation in progesterone levels in 300 mg/kg/day Sprague-Dawley rats only. Mean estrous cycle duration was elevated in treated groups compared to concurrent controls, significant only in Sprague-Dawley rats. Sprague-Dawley rats had a dose-related and significant increase in the percentage of cycle in estrus and a decrease in the percentage of the cycle in diestrus. In contrast, F344 rats had a slightly opposite trend. These differences were consistent with longer term studies, in which Sprague-Dawley rats administered high doses of atrazine spent larger portions of the time in estrus than untreated rats, a phenomenon not seen in F344 rats. Aldous, 10/3/96.

Hormone Receptor Binding Studies

SUMMARY: Studies below show that atrazine is capable of binding weakly to estrogen receptors, and a few estrogen-mediated processes are altered by atrazine in *in vivo* studies. *In vitro* studies evaluating atrazine receptor binding typically have found little or no direct agonist or antagonist activity of atrazine. Several *in vitro* studies showed that atrazine did not alter estradiol-mediated effects on cell growth or activity (see especially Record No. 146008). Investigators concluded that apparent estrogen effects influenced by atrazine are not mediated by direct estrogen-receptor interactions. Aldous, 4/1/98.

220-371 137864 Tennant, M. K. *et al.*, "Atrazine and simazine: possible antiestrogenic properties of chloro-s-triazines in rat uterus," *J. Toxicol. Environ. Health.* 43:183-196 (1994). At least some of these studies were performed at Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC. (1) **Effects on uterine weight:** Ovariectomized Sprague-Dawley rats were dosed with graded doses of atrazine, simazine, or the common metabolite, DACT, for 3 days. On days 2 and 3, half of each group was injected with estradiol.

All rats were killed 24 hr after the final dose. Dose-related decreases in uterine weights were obtained in rats with estradiol treatment plus atrazine. Without estradiol treatment, atrazine had no consistent effect on uterine weight. (2) **Thymidine uptake in uterine tissues:** Intact, immature female rats were gavaged with 0, 1, 10, 50, 100, or 300 mg/kg/day atrazine, simazine, or DACT for 2 days. On day 2, all animals received estradiol injections. After 24 hr, all animals were killed, and uterine slices were prepared for incubation with [3H]-thymidine. Radiolabel incorporation into slices was decreased at 50 to 300 mg/kg/day only (NOEL for this effect was 10 mg/kg/day for all 3 compounds). (3) **Uterine progesterone receptor binding:** Ovariectomized rats were dosed for 2 consecutive days with 50 or 300 mg/kg/day of atrazine, simazine, or DACT: each dose was followed by sc injections of estradiol. Parallel groups were treated with 0 or 300 mg/kg/day of respective triazines, without estradiol. Rats were killed 24 hr after the last dose, and uteri were dissected, homogenized, and supernatants were collected following centrifugation at 36,000 x g for 60 min. The synthetic progesterone ligand, [3H]-R-5020, was used to evaluate progesterone receptor binding. Net progesterone receptor binding was reduced significantly in atrazine and simazine groups subjected to estradiol pretreatment, and non-significantly in estradiol-pretreated DACT rats: in all cases, only at the 300 mg/kg dose level. In studies without estradiol pretreatment, lesser but nevertheless statistically significant reductions in progesterone receptor binding were observed. **Investigators' conclusions:** These studies suggested that the triazines display very low antagonistic potency against estradiol function. It was postulated that these triazines may operate through cellular interactions unrelated to these hormonal effects. Aldous, 7/31/95 (only highly reduced data were presented, hence no worksheet).

220-372 137866 Tennant, M. K. *et al.*, "Chloro-s-triazine antagonism of estrogen action: Limited interaction with estrogen receptor binding" *J. Toxicol. Environ. Health* 43:197-211(1994). This is principally a report of estrogen binding studies as described in Record No. 137859 [Eldridge, J. C. *et al.*, above (part 3)]. In this report, competition studies employing atrazine, simazine, and DACT are reported together. All three test substances led to the same conclusion: the triazines evidently bind only weakly to estrogen receptors, and other molecular interactions must explain part of triazine effects on target tissues. Aldous, 8/1/95 (only highly reduced data provided, hence no DPR worksheet).

220-426 146008 This record is a draft of the article: Connor, K., J. Howell, I. Chen, H. Liu, K. Berhane, C. Sciarretta, S. Safe, and T. Zacharewski, "Failure of Chloro-S-triazine-Derived Compounds to Induce Estrogen Receptor-Mediated Responses in Vivo and in Vitro," *Fundam. Appl. Toxicol.* 30, 93-101 (1996). Study was done at Dept. of Veterinary Physiology and Pharmacology, Texas A&M University, and Department of Pharmacology and Toxicology, Univ. of Western Ontario, London, Ontario, Canada. S-D female rats, 21 days old, received 50, 150, or 300 mg/kg/day atrazine or simazine for 3 days for *in vivo* studies and were sacrificed 20 hr after the third dose. Uterine weights were not significantly changed, however both compounds significantly reduced cytosolic progesterone receptor binding and uterine peroxidase activity. The triazines had no significant effect on estradiol (E2)-induced increases in uterine weights, however there were small (possibly treatment-related) decreases in cytosolic progesterone receptor binding and uterine peroxidase activity in triazine-treated E2-induced rats. In studies with MCF-7 human breast cancer cell line preparations, the triazines had no effect on cytosolic progesterone receptor-DNA complexing with or without E2 addition. Basal MCF-7 cell growth was not affected by the triazines, nor did triazines inhibit such growth when stimulated by E2. Also, when MCF-7 cells were transfected with an estrogen receptor chimera and with a luciferase reporter gene under conditions in which E2 demonstrated a many-fold luciferase activity response, there was no effect by either of the triazines over a wide dosage

range. Co-treatment of triazines with an effective dose of E2 in this assay showed no influence of triazines on the luciferase response. An *S. cerevisiae* strain that is uracil dependent except when the URA3 gene is expressed (an estrogen receptor-dependent gene) underwent measurable growth in the presence of E2, but not with either of the triazines. Results were interpreted to indicate that triazine effects on hormonally-mediated tissues are not mediated by the estrogen receptor. Useful ancillary study (not relevant to data gaps). No adverse effects are indicated. Aldous, 10/3/96.

220-373 137867 Safe, S. et al., "Failure of atrazine and simazine to introduce estrogenic responses in MCF-7 human breast cancer cells." Studies were performed at Texas A&M Univ. and/or Univ. of Western Ontario, submission dated 3/13/95. The two triazines did not compete with TCDD for rat liver cytosolic Ah receptor. Triazines did not elicit estrogenic or antiestrogenic effects, such as estrogen-dependent cell growth. There was no effect on estrogen-dependent nuclear progesterone receptor levels. The triazines did not alter background measurements in the absence of estrogen, nor did they alter the pharmacologic effects of estrogen. Aldous, 8/1/95, (only highly reduced data provided, hence no DPR worksheet).

220-492 156971 McDougal, A., Wilson, C., and Safe S., "Induction of estradiol 2-hydroxylase activity in MCF-7 human breast cancer cells by pesticides and carcinogens," Environ. Toxicol. Pharmacol. (accepted for publication: no volume number was assigned: date of submission of registrants' cover page was 6/26/97). Study was performed at Texas A and M University. Atrazine and several other unrelated chemicals were incubated with MCF-7 cells for 2 or 48 hours in media containing [2-³H]estradiol. Estradiol 2-hydroxylase activity was assayed by counting tritium in the supernatant after removal of residual [2-³H]estradiol by charcoal adsorption. Atrazine, *p,p'*-DDE, and especially DMBA inhibited estradiol 2-hydroxylase activity, whereas several other test articles increased 2-hydroxylase activity over untreated controls. There was no apparent predictive value relating a chemical's effects on 2-hydroxylase to mammary oncogenicity potential. This is potentially useful information, but not applicable to data requirements. Aldous, 6/1/98.

220-217 128787 Wittliff, J. L., "Chronic toxicity study in rats: Influence of atrazine ingestion on steroid-receptor recognition," Hormone Receptor Laboratory, University of Louisville, 10/15/90. Rats from a reproduction study (see Record No. 063785) were maintained chronically at 0, 10, 50, or 500 ppm atrazine and evaluated for changes in various sex hormone receptor binding effects in mammary gland, pituitary gland, and uteri. Interim sacrifices were at weeks 8 (males and females) or 35 (females). Terminal sacrifices were at week 52 (males) or 104 (females). A subset of lifetime study females (10 controls and 10 high dose group) was taken off treatment at wk 65. Estrogen and progestin in vitro binding to cytosol receptors was measured, as well as prolactin binding to membrane receptors. The specific binding capacity (SBC) and binding constants (K_d) were compared for uterine tissues, and for "normal" and "tumor-type" tissues of mammary and pituitary glands as a function of atrazine exposure. The SBC's for estrogen and progestin were increased significantly in tumor tissues. This study did not identify atrazine effects. Standard deviations were often large compared to means, so that this study did not offer potential to identify subtle changes. Aldous, 10/3/96.

Chronic Study in F1 Treated Rats Derived from a Reproduction Study:

220-446 147021 Rudzki, M. W., G. C. McCormick and A. T. Arthur, "Atrazine Technical: Chronic toxicity study in rats," Study No. 852214, Research Department, Pharmaceuticals

Division, Ciba-Geigy Corporation, Summit, NJ, 1/28/91. Crl:VAF/PlusJCD7(SD)BR rats were used in a reproduction study, (DPR Record No. 063785; Ciba-Geigy Study No. 852063), and F1a offspring from that study were randomly selected from respective groups for the present study. Initially there were 50/sex/group at 0, 10, 50, and 500 ppm in diet. Males of all groups were sacrificed at wk 8 (10/dose) or 52 wk (all remaining males). Females (excepting those assigned to a crossover study) were sacrificed at wk 8 (10/dose), or wk 35 (10/dose), or wk 104 (20/dose at 0 and 500 ppm, 30/dose in other groups). Control and 500 ppm females assigned to the crossover study (10/group) were maintained on original test diets for 65 wk, then switched from 0 to 500 ppm or vice versa for the rest of the 104 wk study. Primary parameters evaluated were histopathology of mammary gland (both sexes), testes, and pituitary (females only). Pituitary glands were also treated with immunocytochemical stains for prolactin, FSH, and LH. Other parameters evaluated included b.w., food consumption, hematology, clinical chemistry, and ophthalmology. Apparent NOEL for parameters evaluated = 50 ppm (body weight decrements in both sexes, and modest reductions in RBC parameters and elevations in cholesterol levels in females). Males had no indication of histopathologic change. There was an apparent increase in pituitary adenomas in high dose females, and also in females in the two crossover studies. This appears to have been due to unusually low incidence in 0 and 50 ppm females compared to historical control values, rather than a treatment effect. No adverse effects were identified, however this study did not provide major new information relating to previously identified oncogenicity of atrazine. Value of information was limited due to the small group sizes. Study is acceptable as an ancillary study, given these limitations. Aldous, 10/3/96.

Unacceptable Studies, Not Usable for Hazard Assessment

014 942527-8 (1961, Hazleton Labs) No toxicity seen. UNACCEPTABLE study: dosages too low, inadequate histology, test article apparently not technical material, doses changed mid-study, etc. J. Christopher, 3/5/85.

061 039985 (1981, IBT) Summary only. Appears to be the study classified as Core Supplementary data in a 1983 Dynamac report (p. 2 of EPA 1-liners). J. Schreider, 4/18/86.

053 016906 Exact duplicate of 061 039985

EPA one-liner: "IBT-supplementary; Dynamac Corp.; Contract # 68-01-6561; accepted by EPA 5/31/83. (Two year feeding; IBT # 622-06769)." This is probably the study in Vol. 061, Record #039985, mentioned under "Chronic, Rat," earlier in this summary.

095 059080 "Interim Report on Atrazine Chronic/Oncogenicity Feeding Study IARC," (National Institute of Hygiene, Budapest, Hungary.) This was a summary statement only: **not a complete study**. Atrazine technical grade (batch No. 0041424; 98.9%) was administered in the diet at 500 and 1000 ppm for 8 weeks to Fischer F344/LATI rats (51-56 rats/group). Later, the levels were lowered to 375 and 750 ppm due to toxic signs. Body weight, food and water consumption were regularly monitored. At death, animals were completely autopsied and histology was performed on tissues. **Adverse effect indicated.** Dose-related increase in combined leukemia/lymphoma incidence was observed in males (not statistically significant) and in females (statistically significant). A significant increase in benign mammary tumors in males at 1000 ppm and a statistically significant increase in infiltrating uterine adenocarcinomas (dose related) in females was observed. M. Silva, 11/20/87. (A more detailed report is found in Record No. 069686: see 1-liner under oncogenicity, rat, below).

220-112 069686 Wetzel, L.T. "Review of a Hungarian Institute of Hygiene atrazine toxicology study," performed at Hungarian Institute of Hygiene in Budapest under auspices of WHO. Wetzel's evaluation was dated 1/13/87. This was the Institute's first long-term oncogenicity study. Atrazine (98.9%) was fed to 55/group to F344 rats at final dose levels of 0, 375, or 750 ppm until natural deaths of the animals. Males had increased incidence of combined mammary tumors (incidence of 1, 1, and 8 in increasing dose groups). The finding was not considered by investigators to represent a treatment effect, because 6 of the 8 high dose tumor-bearing animals died after the last control male had died, hence preferential survival had biased the incidence. (Survival of control males was very poor, with 100% mortality at about week 110). There was no increase in mammary tumors in females. Study does not appear to offer meaningful data, even after finalization of the report. Aldous, 10/18/93.

220-540 170886 Liu, C. Y. and A. K. Thakur, "Statistical report for survival and mammary tumor analyses from the Fischer 344/Lati rat study (Pinter et al., 1990)." This submission, dated Sept. 2, 1999, was prepared in response to a request by a U.S. EPA reviewer for clarification of study details. The most complete evaluation of this study provided to DPR is Document No. 220-112, Record No. 069686: an evaluation by L. T. Wetzel, entitled "Review of a Hungarian Institute of Hygiene atrazine toxicology study" dated 1/13/87 (above). The present submission provides individual data on mammary tumors for males and females, plus some statistical analyses. Incidence data of concern (mammary tumors in males) are consistent with results previously reported. Results do not lead to change in acceptability or "possible adverse effect" status from previous submissions. Aldous, 10/15/99.

Interpretative Writings by Investigators (generally without substantial new data)

220-541 171843 Breckenridge, C., J. McFarland, and J. Stevens, "Summary of Atrazine's mode of action in the female Sprague-Dawley rat." Addendum to 220-511:160554, 10/29/99. This summary, by Novartis Crop Protection, Inc., reviewed several themes, none of which are unique to this submission, considered to support treatment of atrazine as a threshold toxicant. The first tab provides several figures and tables relating to major studies previously reported by registrants, and several published studies. Additional ongoing studies were identified in this section. The second tab is an analysis by JSC Sielkin (authors: Sielkin, R. L., C. Valdez-Flores, and L. Holden,) entitled "Palpable tumors in Sprague-Dawley rats: time-to-tumor analyses." Investigators evaluated 35 predictor variables using Multistage-Weibull time-to-tumor analyses with respect to mammary adenocarcinoma and fibroadenoma incidence in Record No.160554. Investigators concluded that fibroadenoma had a very strong correlation with the parameter, "mammary secretory" activity. Additional parameters correlated with fibroadenoma were mammary galactoceles, pituitary adenoma, and number of abnormal days in diestrus during weeks 1 to 26. The executive summary of this record stated that "high level stimulation of the mammary gland with prolactin has been shown to be linked to the development of fibroadenoma as a result of ductal enlargement, lobulo-alveolar development, the development of secretory activity and the formation of milk cysts." The data in Record No.160554, however, do not show a positive association between mammary secretory activity and fibroadenoma at any of several selected time periods in the study, as shown in the DPR review. The same investigators' analyses of adenocarcinoma correlates reported the strongest association to be with "number of observed abnormal estrous days in weeks 5 to 26," followed by "ppm." The latter is consistent with previous DPR review conclusions, but was not analyzed by DPR in response to this submission. DPR would particularly welcome a response to the above concern about the

relationship between fibroadenoma and mammary secretory activity, as well as reviewable data on the effects of GnRH on the LH surge in rats. Aldous, 1/4/00.

220-365 137858 Stevens, J. T. et al., "Hypothesis for mammary tumorigenesis in Sprague-Dawley rats exposed to certain triazine herbicides," J. Toxicol. Environ. Health 43:139-153 (1994). Atrazine and simazine, and to a lesser extent two other chloro-s-triazines, propazine and terbuthylazine, elicited increases in mammary tumors in female Sprague-Dawley rats in previously completed studies (the atrazine study cited was DPR Record No. 044294). Data from three 2-thiomethyl-s-triazines and two methoxy-s-triazines were presented: these tended to be, at best, weak inducers of mammary tumors. There was no consistent pattern of effect on any class of substituted triazines on pituitary tumors. Limited hormone level data (week 104 observations only) indicated marked changes in hormone profiles in aged females. Investigators concluded that some of these triazines elicited tumors by altering hormonal levels over time, however this report did not give supporting information. Aldous 7/27/95 (no worksheet).

220-366 137859 Eldridge, J. C. et al., "Factors affecting mammary tumor incidence in chlorotriazine-treated female rats: hormonal properties, dosage and animal strain," Environmental Health Perspectives 102:29-36 (1994). Reviewed in draft form as 220-172:113603 by Aldous on 10/14/93. Report presents plots and bar graphs, but no individual data. Major findings were as follows. **(1) Effects on uterine weights:** Ovariectomized rats [strain was not specified: judging from a similar publication by the same group (Tennant, M. K. et al., J. Toxicol. Environ. Health 43:183-196 (1994), DPR Record No. 137864), strain was probably Sprague-Dawley] were dosed with 0, 20, 100, or 300 mg/kg/day atrazine for 2 days. On day 2, half of each group was injected with estradiol. All rats were killed 24 hr later. A dose-related decrease in uterine weights was obtained at the 100 and 300 mg/kg/day dose levels in rats with estradiol treatment: without estradiol treatment, atrazine had no effect on uterine weight. **(2) [3H]-thymidine binding in uterine slices from treated rats:** Intact, immature (23-day old) female rats were gavaged with 0, 1, 10, 50, 100, or 300 mg/kg/day atrazine for 2 days. On day 2, all animals received estradiol injections. After 24 hr, all animals were killed, and uterine slices were prepared for incubation with [3H]-thymidine. Radiolabel incorporation into slices was decreased at 50 to 300 mg/kg/day only. **(3) Binding studies in uterine cytosol:** A competitive binding study was similar to a simazine study (Record No. 128788, summarized toward the end of this Summary of Toxicology Data). Initial attempts to study competitive binding of atrazine to the estrogen receptor in uterine cytosol were futile: no attainable atrazine concentration was sufficient to measurably displace estradiol when atrazine and estradiol were applied simultaneously. Pre-incubation of 0.1 mM atrazine with cytosol at RT, followed by chilling the preparation prior to addition of 3 nM labeled estradiol, led to appreciable but reversible reduction in estradiol binding. Investigators concluded that atrazine possessed a very weak inhibition of estradiol, possibly with competitive and noncompetitive components. The inhibition seen in vitro was considered to be plausibly relevant to effects seen in high dose levels in chronic studies. **(4) Vaginal cytology studies to evaluate estrus cycles:** Investigators discussed data reviewed under Record Nos. 123799 and 123801: analyses of vaginal cytology over time in chronic studies using Sprague-Dawley and F-344 rats that suggested that the Sprague-Dawley females dosed with high amounts of atrazine tended to have elevated estradiol levels and greater percentage of time in estrus, presumed to enhance mammary tumor yields in affected strains. **(5) Hypothalamic histopathology:** The arcuate nucleus of the rat hypothalamus is known to influence estrous cycles. The di-dealkylated chlorotriazine metabolite, diaminochlorotriazine (DACT) was shown to cause premature formation of inclusion granules of astrocytes in that nucleus (EM evaluation of hypothalamic tissues following dosing

of 0 or 1000 ppm DACT for 20 to 48 wk). These inclusions are part of a normal aging process, which has been shown to be blocked by ovariectomy, and enhanced by chronic estrogen administration. Thus, study results suggest a possible central mode of action for atrazine-like molecules. **Investigator conclusions:** differences between humans and rodents relating to the process of reproductive aging and to the physiological nature and hormonal responsiveness of mammary tumors were presented as reasons why triazines may not be as serious a health concern to humans as might be inferred from rodent studies. Aldous, 7/28/95 (only highly reduced data were presented, hence no worksheet).

220-172 113603 Draft of the publication in Record No. 137859, above.

220-367 137860 Stevens, J. T., "Rat mammary tumorigenesis: Relevance of hormonal imbalance to dose selection," (published in The Toxicology Forum, The Given Institute of Pathobiology, Aspen CO, July, 1994). This is principally a risk assessment document, recommending that tumors associated with atrazine be regulated on the basis of a NOEL for chronic effects associated with hormonal imbalance, rather than by extrapolation of tumor incidence curves to the origin. Major points are differences between strains of rats (i.e., Sprague-Dawley females have protracted estrus associated with elevated estradiol upon aging, compared to F-344 rats, which are characterized by extensive proestrus and elevated progesterone in geriatric life). The author contends that the doses that produce highly significant elevations in tumor incidence (i.e. $p < 0.01$), substantially exceed the MTD, based on body weight gain criteria. Aldous, 8/1/95 (only highly reduced data provided, hence no DPR worksheet).

220-369 137862 Wetzel et al., "Chronic effects of atrazine on estrus and mammary tumor formation in female Sprague-Dawley and Fischer 344 rats," J. Toxicol. Environ. Health., 43:169-182 (1994). This is a review of some of the information submitted in Record No. 123802 and related records. No DPR review is appropriate. Aldous, 7/31/95.

220-456 151471 Breckenridge, C., "Summary of additional comments on the response to the Special Review Position Document 1 for pesticide products containing atrazine and simazine: Supplement II" 10/30/96. This document summarizes main points associated with risk assessment. It contains brief reviews of toxicology studies, atrazine metabolism, human exposure assessment, and human safety and ecological risk evaluations. No new studies; no worksheet. Aldous, 3/18/98.

NOTE: In response to a U.S. EPA Special Review on atrazine, registrants have organized a "Consensus Panel," which has convened on several occasions. The panel has gathered data to highlight new findings about the mechanism by which atrazine elicits mammary tumors in female Sprague-Dawley rats, to discuss physiological differences between test animals and humans, and to advocate "margin of exposure" rather than linear extrapolation techniques to address human risk. Some studies cited here, particularly those by Tyrey *et al.* and Cooper *et al.* have not been submitted to DPR, although they may contribute to the evaluation of mechanisms of reproductive risks. "Consensus Panel" reports are summarized below. Aldous, 3/18/98.

220-460 151479 [Consensus Panel] "Weight of the evidence on the oncogenic potential of atrazine: Consensus Panel report," 3/21/95. This appears to be the first of 3 Consensus Panel reports available to date. This report reviews several major atrazine chronic rodent studies, and summarizes mammary tumor findings associated with several analogs of atrazine. There are no new data requiring DPR review (no worksheet). Aldous, 3/18/98.

220-459 151477 Simkins, J. W., "Evaluation of a hormonal mode of action for mammary carcinogenesis of the chlorotriazine herbicides: second consensus panel report," 10/30/96. This report cites data by Cooper *et al.*, 1995, which have not yet been submitted to DPR. There are no fundamentally new insights on atrazine (no worksheet). Aldous, 3/18/98.

220-491 156970 Simkins, J. W., "Evaluation of a hormonal mode of action for mammary carcinogenesis of the chloro-s-triazine herbicides: third consensus panel report," 6/30/97. This panel analysis cites many studies relating to mechanism of atrazine-elicited Sprague-Dawley female rat mammary tumor responses. Some cited studies are not yet received by DPR, and some information on a chronic supplemental study involving ovariectomized rats (DPR Record No. 151475) is more recent than the interim report presently available. This report does not indicate fundamental changes from previous conclusions based on studies in progress. Page 19 of this report suggests that a study (possibly Tyrey *et al.*, 1996) has found that atrazine does not affect pituitary responsiveness to GnRH. That study, and all other studies relating to the disposition of hormones related to reproduction should be submitted to DPR. Aldous, 3/18/98.

220-374 137869 Simkins, J. W., "Evaluation of a hormonal mechanism for mammary carcinogenesis of the chlorotriazine herbicides: Consensus panel report," 3/14/95. A panel of an independent group of scientists determined that atrazine is neither genotoxic nor intrinsically estrogenic, however atrazine appears to accelerate reproductive senescence (as maintained by other Ciba documents). The panel recommended several new studies, for which the protocols are given in another record (220-394 137917, under "Proposed Protocols," above). No reviewable data. Aldous, 7/30/96.

220-395 137928 [Consensus Panel] "Weight of the evidence on the oncogenic potential of simazine: Consensus panel report," 3/21/95. Conclusions were essentially the same as for atrazine (Record No. 137869, above). No reviewable data. Aldous, 7/30/96.

220-375 137870 Wetzel, L. T. and M. O. Tisdell, "Ciba response to May 3, 1994 letter by Penelope Fenner-Crisp, Director, Health Effects Division, Office of Prevention, Pesticides and Toxic Substances, U.S. EPA," 3/20/95. Primary text is a response to a letter by Dr. Fenner-Crisp, who referred in large part to data presented in a Nov. 1993 meeting with U.S. EPA, much of which data are embodied in an analysis by R.F. McConnell (dated 3/10/95, submitted as DPR Document No. 220-376, Record No. 137871, for which a DPR worksheet has been prepared. The letter stated that the etiology of human mammary carcinogenesis is not sufficiently understood to definitively state that the F-344 rat is a better surrogate than the S-D rat. Several possible flaws in the Ciba data were noted, including large SD's in hormone assay means, difficulty in conducting an experiment that could standardize the segment of proestrus in which rats were sampled (considering the large estradiol drop from early to late proestrus), technical problems with reading and interpreting air-dried vaginal smear samples, lack of plausible consistency in estrous cycle staging over the time period of 9 to 18 months, lack of estrous stage sampling between 3 and 9 months (a critical time period if atrazine is shortening the period before which S-D females attain constant estrus), and certain concerns about Ciba interpretation of hormone level data. Ciba authors defend data integrity and maintain that data are consistent with the hypothesis that atrazine works by modulating the LH surge, which is critical to maintenance of estrous cycling in S-D rats. No worksheet is relevant for the present record, however see review of the cited record (No. 137871). Aldous, 7/31/96.

CHRONIC, DOG

**** 099, 129 062676, 087668** O'Connor *et al.*, "Atrazine Technical: 52-week Oral Feeding Study in Dogs," (Ciba-Geigy Corporation, 10/27/87). Atrazine Technical (Batch FL 850612, purity = 97%), was fed in the diet to Beagle dogs at 0 (6 dogs/sex/group), 15 and 150 (4 dogs/sex/group) and 1000 ppm (6/sex/group) for 52 weeks. NOEL = 15 ppm (increased mortality at 150 and 1000 ppm, decreased food consumption and body weight gain at 1000 ppm; decreased RBC, Hb, HCT, total protein and albumin as well as increases in platelet counts, phosphorus, sodium, glucose and liver and ovary relative weights at 1000 ppm). **Possible adverse effect.** At 150 and 1000 ppm, females experienced increased heart weights and in both sexes treatment related electrocardiographic changes in the heart accompanied by gross and histologically detectable pathology were observed. Previously reviewed as having a NOEL of 15 ppm (Silva, 5/20/88), the study has been re-evaluated based upon information submitted to DPR by Ciba-Geigy. The status, however, remains unchanged. M. Silva, 11/29/89.

220-099 062676 This record was previously reviewed by DPR. This review addresses a difference between DPR and U.S. EPA on the NOEL for this study. U.S. EPA considers the NOAEL in the primary dog chronic study to be 150 ppm (4.97 mg/kg/day: see <http://www.epa.gov/IRIS/subst/0209.htm>), instead of 15 ppm, as determined by the Cal-EPA reviews by M. Silva (5/20/88 and 11/29/89). The present examination by Aldous includes a supplemental worksheet tabulating histopathology and EKG data from the subject study. A histopathology table provided in this re-examination shows that there is no continuum of findings in heart from 150 to 1000 ppm. In general, the locations of tissue pathology for these two treatment groups are different, and the lesions are fundamentally different. The single 150 ppm decedent, the only dog in that group with noted heart histopathology, had primarily inflammatory responses with associated necrosis. The high dose dogs demonstrated myolysis and focal atrophy, restricted primarily to atrial myocardium. The EKG amplitudes designated as the P-II measurements (height of P-wave in mv) had been considered to reflect a treatment effect at both 150 and 1000 ppm in the 1988 and 1989 DPR reviews. The P-II data were reported in increments of 0.1 mv. Common values were 0.2 and 0.3 mv, and rarely 0.4 mv. Values of 0.0 or 0.1 mv were only observed in high dose dogs, which demonstrated marked reductions in P-II. The P-II values in 15 ppm or 150 ppm dogs of either sex were comparable to controls. In contrast, markedly reduced amplitude of the P-wave in 1000 ppm dogs appears to be consistent with the characteristic myocardial atrophy and myolysis. Thus treatment-related EKG changes and histopathology findings were limited to the 1000 ppm males and females, and nearly all dogs of this treatment group were involved. Absolute heart weights were non-significantly elevated in 150 ppm males, and were statistically significantly elevated in 150 ppm females, prompting a post-hoc comparison of heart weights in the present study with those of 5 other chronic and subchronic studies involving atrazine, simazine, or analogs: a short worksheet entitled "Triazine chronic and subchronic heart weights" provided as an appendage to this review. There were no consistent treatment effects on absolute or relative heart weights nor on body weights in the range of 150 ppm or less for atrazine or simazine, nor for related triazines, insofar as these were evaluated in the range below 500 ppm. High dose responses to the triazines commonly led to decreased body weights and decreased heart weights. In summary, the U.S. EPA determination of a NOEL of 150 ppm is more supportable than the lower NOEL of 15 ppm currently utilized by DPR. This supplementary worksheet was based on analysis originally provided for DPR staff preparing an SOT poster presentation in 2005. C. Aldous, 1/25/08.

220-0542 172597 and 172598 This is a copy of a letter from Novartis to DPR dated Jan. 7, 2000, plus the U.S. EPA DER with stamped date of 12/19/89, revising the EPA's dog chronic NOEL for reasons consistent with the above paragraph (new 1-liner on re-examination of dog

chronic study 220-099 062676). DPR assigned two record numbers to this Novartis letter plus U.S. EPA DER, because Novartis disputed the definitive chronic NOEL assigned by DPR, to which chronic effects of two species (rat and dog) might apply. Aldous, 1/25/08.

220-049 016883 (Woodard Research Corp., 1964) 3/sex/group; summary. See EPA one-liner. Two-page summary noted tremors and stiffness of hindlimbs at 1500 ppm (HDT). Lacrimation at 15-1500 ppm. Findings **not considered as significant adverse effects** requiring risk assessment. J. Schreider 5/09/86.

EPA one liner: "Systemic NOEL = 150 ppm; systemic LEL = 1500 ppm (LDT) [sic: should read HDT] reduced food intake, decreased body weight, reduced Hb and hematocrit values. Levels tested = 0, 15, 150, 1500 ppm. Core grade: Supplementary. (Two-year feeding - dog; Woodard Research Lab, 1964; MRID # 00059213)." As of 10/1/93, U.S. EPA had down-graded this study to "invalid," considering multiple serious deficiencies (see U.S. EPA IRIS document on Atrazine at <http://www.epa.gov/IRIS/subst/0209.htm>). Aldous, 8/86, with edits citing current EPA disposition on 2/7/07.

220-0563 180553 This is a 57-page report of the Woodard study summarized in 220-049 016883, above. It contains individual data for many aspects of the study, but not for clinical signs. NOTE: A re-examination of the subsequent accepted dog chronic study (220-099 062676) found no lacrimation in the clinical signs table at dose levels up to 1000 ppm. This further confirms that the invalid 1964 Woodard Research Corp study has no potential usefulness for toxicity assessment (Aldous, 11/16/07).

ONCOGENICITY, MOUSE

** 100 064254 "Oncogenicity Study in Mice," (Ciba-Geigy Corporation, NJ, 10/30/87). Atrazine Technical (Batch FL # 841802; purity = 97.6%) was administered in the diet at 0, 10, 300, 1500 and 3000 ppm to 60 CD-1 mice/sex/group for 91 weeks. NOEL = 10 ppm (increased mortality in females at 3000 ppm; decrease in body weight gain at 300, 1500 and 3000 ppm, decrease in water and food consumption at 1500 ppm in males and 3000 ppm in both sexes; reductions in RBC, HCT and Hb at 1500 ppm in males and 3000 ppm in both sexes; decrease in absolute brain weight at 3000 ppm in both sexes and decreases in mean absolute kidney weight at 3000 ppm in females; cardiac thrombi incidence was increased in 1500 ppm females and 3000 ppm in both sexes). No adverse effect. No oncogenic effects were observed with atrazine under the conditions of this study. ACCEPTABLE. M. Silva, 5/20/88.

220-407 138980 One page of historical control data for the above mouse oncogenicity study. Data were provided on U.S. EPA request. No DPR review.

014 942529 (Bionetics Research Labs, 4/29/69) NCI journal article. Study not upgradeable: insufficient information to make meaningful judgement. No individual necropsy/histology data. Too few animals. Too few doses. No indication of adverse effects noted. J. Christopher, 3/5/85.

061 039986 (1969, NCI) One-sentence summary, apparently referring to 014 942529. J. Schreider, 4/18/86.

053 016904 Exact duplicate of 061 039986.

061 039987 (IBT, 1981) Summary. No adverse effects reported. See EPA one-liner, below. J. Schreider, 4/18/86.

EPA one-liner: (Oncogenic, mice; IBT, #8580-08906; 6/30/81) Study contracted to Dynamac Corporation for review. Classified by Dynamac as “Core Supplementary Data” (hence study not acceptable by EPA to fill data gaps). EPA reviewers accepted the Dynamac judgement, hence replacement study required. 5/31/83.

053 016903 Exact duplicate of 061 039987.

REPRODUCTION, RAT

** 101, 118 063785, 072136 “Atrazine Technical: A Two Generation Toxicity Study in Rats.” (Ciba-Geigy, Summit, NJ, Laboratory Study # 852063, 11/17/87 and 12/14/88) Atrazine technical, Batch FL-841802, 97.6% purity, was administered to 30 CR CD, VAF/PLUS rats/sex/group in the diet at 0, 10, 50 or 500 ppm continuously for 2 parental generations of animals and their offspring throughout all phases of the study. Parental NOEL = 50 ppm (decreased body weight, body weight gain and food consumption in both sexes at 500 ppm). Reproductive NOEL > 500 ppm (no effects on reproduction was observed). No adverse effect. Initially reviewed as unacceptable but upgradeable with submission of missing data on culled pups for F1 and F2 and no necropsy data for F1 pups and only 5/sex/group were given for F2 pups; also noted that P2 generation had only 18 litters at 10 ppm. M. Silva, 5/20/88. Record # 072136 in vol. #118 cites the pages on which the requested data are found in part 6 of 6 in volume 101. These data upgrade the study to ACCEPTABLE status. J. Gee, 2/9/89. A re-evaluation of this study was performed in response to an EPA review stating a reproductive effect in F2 pups at ≥ 50 ppm based upon weight decrease in males at 21 days. CDFA notes the effect at 21, but not 14 days. Therefore, the weight decrease was probably due to increased consumption of treated diet (rather than milk). On a mg/kg basis, the treated diet would be an overdose for the pups. The weight decrease is not considered to be of toxicological importance. The study status remains unchanged. M. Silva, 6/28/90.

014 942530 (Woodard Research Corp., 1966) NOT ACCEPTABLE nor upgradeable. No adverse effects reported. See EPA one-liner. J. Christopher 3/5/85.

EPA one-liner: “Systemic NOEL > 100 ppm (HDT); reproductive NOEL > 100 ppm (HDT); dietary regime altered. Levels tested = 0, 50, 100 ppm. Core grade: Supplementary. (Three-generation; Woodard Research Labs, 1966; MRID #00024471).”

061 039990 (Woodard Research Corp., 1966) Brief summary of 014 942530. J. Schreider, 4/18/86.

053 016900 Exact duplicate of 061 039990.

220-231 128906 Supplement to 101, 118 063785, 072136. U.S. EPA had argued that the NOEL for reproductive effects was 10 ppm, based on a modest decrement in body weights of 50 ppm F2 male pups at day 21 post-partum. This supplement states that the pup weights were not correctly evaluated, and that the pup weight decrements should not have been tagged as statistically significant in the first place. CDFA (now DPR) had placed the reproductive effects NOEL at > 500 ppm (attributing the pup weight changes to direct consumption of diet by rapidly growing pups after day 14). For this reason, this supplemental submission is of unlikely importance to study evaluation. Aldous, 9/3/96.

TERATOGENICITY, RAT

050 016887 "A Teratology Study of Atrazine Technical in Charles River Rats," Ciba-Geigy, 9/8/84. Atrazine technical (96.7%); administered by gavage on days 6 - 15 of gestation to groups of 27 mated rats at 0, 10, 70, or 700 mg/kg/day. Initially reviewed (Parker, 4/30/86) as a possible adverse effect since the Maternal NOEL = 70 mg/kg/day (mortality) while the developmental NOEL < 10 mg/kg/day (increased visceral and skeletal variants). Additional data supplied, 094 54802, included historical control data. Variants were in the range of historical control values; therefore, no adverse effects are indicated and the developmental NOEL = 70 mg/kg/day. Study remains NOT ACCEPTABLE and not upgradeable (excessive mortality, only five litters at high dose). J. Parker, 7/3/87.

094 054802 Ciba-Geigy rebuttal to 016887 includes historical control values.

220-361 137854 Infurna, R. et al. "Teratological evaluations of atrazine technical, a triazine herbicide, in rats and rabbits," J. Toxicol. Environ. Health. 24:307-319 (1988). Publication corresponding to Record No. 16887, reviewed by CDFA in 1987. No separate worksheet is appropriate.

220-407 138991 Ciba response to U.S. EPA regarding purity of atrazine in Record No. 016887, above. Response also provided historical frequency of runted fetuses. No review is necessary for DPR, since fetal body weight was not a noted concern in the review of the above study, and was specifically noted as not evident at the HDT of 100 mg/kg/day in the accepted rat teratology study (Record No. 073682, below). Aldous, 9/4/96.

014 942531 (Ciba Geigy, 1971) Doses of 0, 100, 500, and 1000 mg/kg/day by gavage. Summary and assessment data, tables included. See EPA one liner, below. NOT ACCEPTABLE, not upgradeable. High dosage apparently too high. No individual data. Test article not defined. J. Christopher, 3/5/85.

EPA one-liner: "Maternal NOEL = 100 mg/kg; Maternal LEL = 500 mg/kg (wt. loss); Fetotoxic NOEL = 100 mg/kg; Fetotoxic LEL = 500 mg/kg (fetal resorptions, wt. loss); developmental NOEL > 1000 mg/kg (HDT). Core grade: Minimum, (Ciba Geigy, Switzerland, 1971; MRID # 00038041)."

050 016886 Exact duplicate of 014 942531.

061 039988 (Ciba Geigy, 1971) Summary of 014 942531. J. Schreider, 4/18/86.

053 017902 Exact duplicate of 061 039988.

118 072136 Document providing data for dosing suspensions for the rabbit teratology study (see below) contains a statement that there is an ongoing rat teratology study with Atrazine. The document is signed and dated December 14, 1988. J. Gee, 2/9/89.

** 120, 73682, "Atrazine Technical: A Teratology Study (Segment II) in Rats," (Ciba-Geigy Corporation, Summit, NJ, Project No. 89006, 2/23/89), Atrazine Batch No. FL 841802, 97.6% purity (Document No. 220-101, page 1341), 0.05, 0.25, 1.00% in 3% aqueous cornstarch containing 0.5% Tween 80 dosed at 5, 25, 100 mg/kg/day, vehicle controls 10 ml/kg/day, day 6-15 gestation, dose adjusted for body weights on days 6, 8, and 12; 26 sperm positive

females/dose; no adverse effects; Developmental NOEL = 25 mg/kg/day (delayed ossification without decreased body weight), maternal NOEL = 25 mg/kg/day (decreased feed consumption, body weights, and body weight gains). ACCEPTABLE. (DiBiasio 5/26/89)

220-292 129149 Duplicate of Document No. 220-120, Record No. 73682, above.

220-228 128903 Chemical analysis summary relating to Record No. 073682, above.

TERATOGENICITY, RABBIT

** 050, 118 016885, 072135 “Teratology Study of Atrazine Technical in New Zealand White Rabbits.” (Ciba-Geigy Pharmaceuticals, NJ, 9/18/84 and 12/8/88) Atrazine technical, 96.3%, lot FL-821014; given by oral gavage at 0 (3% corn starch plus 0.5% Tween 80), 1, 5 or 75 mg/kg/day, days 7 - 19 of gestation to 19 New Zealand White rabbits/group; maternal NOEL = 1 mg/kg/day (decreased weight gain and food consumption); developmental NOEL = 5 mg/kg/day (increased resorptions, decreased fetal weight and number of live fetuses). Initially reviewed as unacceptable but upgradeable based on the lack of data on dosing suspensions. J. Parker, 4/30/86. Record # 072135 in 118 contains data on homogeneity, stability and adequacy of preparation of suspensions at the same facility and in the same vehicle. Status is upgraded to ACCEPTABLE with no adverse effect noted. J. Gee, 2/9/89.

061 039989 (Ciba-Geigy). Summary of 050 016885, see above. J. Schreider, 4/18/86

053 016901 Exact duplicate of 061 039989.

220-361 137854 Infurna, R. et al. “Teratological evaluations of atrazine technical, a triazine herbicide, in rats and rabbits,” J. Toxicol. Environ. Health. 24:307-319 (1988). Publication corresponding to Record No. 16887, reviewed by CDFA in 1987. No separate worksheet is appropriate.

220-407 138981 One-page record states purity of atrazine in the above report (Record Nos. 016885 and 072135) was 96.3%. No DPR worksheet.

TERATOGENICITY, MICE

EPA one-liner: “Use of DMSO and insufficient data on litters; fetuses make evaluation impossible. AKRC3H, decreased # of live fetuses, AKR exhibited reduced fetal wt. Core grade: Supplementary. (Bionetics Research Labs, 1968; MRID # 00023558).” DPR does not have this study.

TERATOGENICITY, (GENERAL OVERVIEW)

220-362 137855 Johnson, E.M., “An evaluation and critique of atrazine developmental toxicology safety evaluations and human epidemiological data: a review of published and unpublished studies for hazard potential and risk estimation.” Author concludes that the database supports the conclusion that atrazine does not pose special risks for developmental

toxicity. Since the DPR evaluations of teratology studies to date do not disagree, there is no need for critical review of this document. Aldous, 7/25/95.

GENE MUTATION

** 105 064333 “Salmonella/Mammalian-Microsome Mutagenicity Test,” (Ciba-Geigy, 12/86). Atrazine technical (G 30027, lot no. 210200, purity = 98.3%) was used in a mutagenicity test with Salmonella typhimurium strains TA98, TA100, TA1538 and TA1537, with and without activation at levels of 0 (DMSO = vehicle), 20, 78, 313, 1250, and 5000 µg/0.1 ml DMSO. A toxicity test was also run at concentrations of 0 (DMSO), 0.08, 0.31, 1.2, 4.9, 19.5, 78.1, 312.5, 1250 and 5000 µg/0.1 ml DMSO. Plates were incubated for 48 hours at 37°C. Positive controls were included with and without activation and for the toxicity test. No mutagenicity was observed with atrazine. ACCEPTABLE. M. Silva, 5/3/88.

088 065713 “An Assessment of the Genetic Toxicity of Atrazine: Relevance to Health and Environmental Effects,” (Ciba-Geigy Corporation, 12/87). The objective of this discussion was to 1) reconcile the inconsistencies of test results within the data base, 2) interpret the significance of the possible difference in bioactivation of atrazine in plants and animals and 3) draw a conclusion regarding the possible genetic toxicity of atrazine to humans. The discussion concluded that a) it is not scientifically possible or necessary to reconcile all the test-response conflicts for atrazine, however using the weight-of-evidence system developed by ICPEMC, atrazine is considered non-mutagenic, b) extrapolation of genotoxic activity from the plant activation studies to effects in mammals or mammalian cells has not been documented, c) no case has been adequately established to show that atrazine is a genotoxic agent capable of initiating neoplasia or inducing transmissible mutation in mammalian germ cells. This information is supplementary. M. Silva, 5/20/88.

014 942532 (Natl. Institute of Genetics, 1975) Mass survey of 166 pesticides. No adverse effects indicated. No data. Report NOT ACCEPTABLE. J. Christopher, 3/5/85.

061 039991 (SRI) One-paragraph summary. Salmonella strains TA1535, TA1537, TA1538, TA98 and TA100. Concentrations ranged from 50 to 5000 mg/plate with and without rat liver activation. No adverse effects reported. Report NOT ACCEPTABLE in summary form as submitted: insufficient information for assessment. See EPA one-liner, below. J. Schreider, 4/18/86.

EPA one liner: “Not mutagenic up to 5000 micrograms/plate with and without activation in TA 1535, 1537, 1538, 98, 100. Core grade: Acceptable. (SRI, 1977; MRID # 0006064).”

061 039992 (SRI) Summary - Mouse/Host-Mediated Assay. Salmonella strains TA1535 or TA1538 injected i.p. following oral administration of one or five doses. Atrazine apparently not mutagenic in this system. Report NOT ACCEPTABLE. Insufficient information for independent assessment. J. Schreider, 4/18/86.

061 039993 (Ciba-Geigy) One paragraph summary - Ames. Technical atrazine; Salmonella strains TA98, TA100, TA1535 and TA1537; with and without activation at 0, 10, 30, 90, 270 or 810 µg/0.1 ml/ plate. No apparent mutagenic effect reported. Report NOT ACCEPTABLE. Insufficient information for review. J. Schreider, 4/18/86.

061 040005 (Abstract from Mutagen Society, 1981) Ames assay with water-soluble extracts from corn grown in treated and untreated soils. No adverse effect reported. Summary. Report NOT ACCEPTABLE. Insufficient information for assessment. J. Schreider, 4/18/86.

061 040007 (Drug & Chemical Toxicology, 1984) Summary report, not acceptable. Salmonella TA100 was tested with corn extracts in the reversion assay and TM-677 in the forward mutation assay. Insufficient information for assessment. No adverse effect indicated. J. Schreider, 4/18/86.

EPA one-liner: “No metabolic activation. Procedure not delineated. Cytotoxicity limit improperly chosen, interpretation not acceptable. Core grade: Unacceptable. (Entomology Dept., P.S.U., by K. A. Rashid, MRID # 00079923).” DPR does not have this study.

Summary: While a number of studies have been conducted with atrazine, a number are incomplete reports or are publications as on file at DPR. The rebuttal submitted in Document 220-094 discusses and evaluates a series of studies in all genotoxicity test types. The complete reports need to be submitted to DPR. The data requirement is, however, fulfilled by Record # 064333. Silva, 5/88 and Gee, 2/89.

220-049 A 1-paragraph summary of negative studies. No DPR review.

CHROMOSOMAL ABERRATIONS

** 105 064335 “Dominant Lethal Test, Mouse,” (Ciba-Geigy Ltd, 9/81). Atrazine technical (G 30027, lot 6663) was administered orally (by gavage) in a single dose at 0 (vehicle = 0.5% sodium carboxymethyl cellulose), 444 and 1332 mg/kg to male Tif.MAGf(SPF) mice (20/group). 2-Aminoanthracene at 78 and 234 mg/kg was used as the positive control. Six hours after treatment the males were mated with 2 untreated females. At the end of 1 week, the females were removed from the cages and replaced by another group of 2 females. This procedure was continued for 6 consecutive weeks. Presence of a vaginal plug = “day 0” of gestation. Females were autopsied on the 14th day of gestation. No effects observed at any dose level. The positive control showed a statistically significant increase in the relative number of embryonic deaths at 234 mg/kg in the fourth mating period. No adverse effect. ACCEPTABLE. M. Silva, 5/17/88.

** 181 121283, “Structural Chromosomal Aberration Test Dominant Lethal Test, Mouse, 8 Weeks,” (T. Hertner, Ciba-Geigy Limited, Genetic Toxicology, Switzerland, Laboratory Study No. 911247, 1/7/93). G 30027 technical, purity 97.1%, was given in a single oral dose of 0 (corn oil), 500, 1000, 2000 or 2400 mg/kg to 30 male [Tif: MAGf (SPF)] mice/group. Piloerection and reduced locomotor activity were observed in the three high doses. G 30027 did not induce dominant lethal mutations in male mice; NOAEL = 2400 mg/kg. ACCEPTABLE. (Kishiyama and Gee, 10/15/93)

061 039994 (Ciba-Geigy) Nucleus anomaly test in Chinese hamsters given technical atrazine by oral gavage for 2 days at 0, 282, 564 or 1120 mg/kg/ dose. Bone marrow samples were examined from 3 males and 3 females. No adverse effect noted. Summary. Report NOT ACCEPTABLE. Insufficient information for assessment. J. Schreider, 4/18/86.

061 039995 (Ciba-Geigy) Summary - Chromosomal aberration/Mouse spermatogonia. Male mice were given 0, 444 or 1332 mg/kg for 5 consecutive doses, 8 males per group; 100

metaphases from each mouse were examined. No adverse effect indicated. Report NOT ACCEPTABLE. Insufficient information for assessment. J. Schreider, 4/18/86.

061 039996 (Ciba-Geigy) Summary - Dominant Lethal/Mouse Technical atrazine was given by oral intubation in a single dose at 0, 444 or 1332 mg/kg; number of mice not specified; No adverse effects indicated. Report NOT ACCEPTABLE. Insufficient information for assessment. J. Schreider, 4/18/86.

** 220-408 138993 "Atrazine: Structural chromosomal aberration test: Micronucleus test, mouse" (C. Ceresa, Ciba-Geigy Limited, Switzerland, Lab number 871546, 5/31/88) Atrazine, 98.2% purity, batch 210200, was given by oral gavage to male and female mice, Tif: MAGF, SPF. In the first part, doses were 0 (0.5% carboxymethylcellulose) or 2250 mg/kg, to 24/sex/group. Eight per sex per group were sacrificed at 16, 24 or 48 hours after dosing and slides from five per sex were analyzed for micronuclei in polychromatic erythrocytes. In the second part, 8 per sex per group were treated with 0, 562.5, 1125 or 2250 mg/kg and sacrificed at 24 hours. The positive control in both parts was 64 mg/kg cyclophosphamide with sacrifice at 24 hours. In the first part, a total of 7 females died at the high dose. In the second part, one female died at 1125 mg/kg within the 24-hour period. There was no treatment-related increase in micronuclei formation with any sacrifice time or dose. The positive control was functional. **No adverse effect** under test conditions. **Acceptable.** (Gee, 8/21/96.)

DNA DAMAGE

** 105 064334 "Autoradiographic DNA Repair Test on Rat Hepatocytes," (Ciba-Geigy, 2/9/84). Atrazine technical (purity = 98.2%; batch no. P 210200) was used on rat hepatocytes in vitro at 1.2, 6, 30 and 150 mg/ml medium (4 cultures/group). Cells were exposed for 5 hours. Dimethylnitrosamine (100 mM) served as a positive control. Vehicle (ethanol) and untreated groups served as negative controls. No adverse effect. No effects were observed at any dose. At 150 mg/ml a precipitate was observed. Positive controls were within their historical range. ACCEPTABLE. M. Silva, 5/16/88.

061 040002 (Mutation Research, 1978) Very brief summary. Saccharomyces and Aspergillus nidulans tested for mitotic gene conversion with mouse liver microsomal activation. No adverse effect indicated. Report NOT ACCEPTABLE. Insufficient information for assessment. J. Schreider, 4/18/86.

053 016893 Exact duplicate of 061 040002.

061 040006 (Environ. Mutagenesis, 1982) Saccharomyces cerevisiae tested for gene conversion with corn plant extracts. Insufficient experimental control to identify compound-related effect. No adverse effect indicated. Report NOT ACCEPTABLE. J. Schreider, 4/18/86.

EPA one-liner: "No activation was made. The spot test is limited in usefulness. Core grade: Unacceptable. (T4 Bacteriophage systems; Battelle Memorial Inst., 1977; MRID 00025376)." DPR does not have this study.

EPA one-liner: "Not a mutagen inducer in both rec- assay and reversion assay utilizing H17 Rec+ and M45 Rec- strains of *Bacillus subtilis*, *Escherichia coli*, and *Salmonella typhimurium*. Core grade: Minimum, (Shirash, et. al., Mutagenicity. Research, 1976)." DPR has a very brief summary.

** 220-238 128913, "Atrazine, Autoradiographic DNA Repair Test on Rat Hepatocytes, Tests for Other Genotoxic Effects," (Th. Hertner, Genetic Toxicology, Ciba-Geigy Limited, Basel, Switzerland, Report # 911246, 14 April 1992). The test article was atrazine technical with 97.1% purity. Primary hepatocytes from male Tif: RAIf(SPF) rats were exposed in quadruplicate to concentrations of 0 (DMSO), 15.5, 46.4, 139.2, 417.5, 835.0, and 1670.0 mg/ml for 16 to 18 hours. Unscheduled DNA synthesis was measured by autoradiography. **No increase in unscheduled DNA synthesis** under test conditions. **Acceptable.** (H. Green and Gee, 8/16/96).

220-014 046274 Shirasu, Y. et al., "Mutagenicity Screening of Pesticides in the Microbial System," Mutation Research 10:19-30 (1976). Atrazine was among 166 pesticides screened for mutagenicity endpoints, and was negative for the rec- assay in B. subtilis. Point mutation assay in the same publication was given record No. 942532 (also negative). No DPR worksheet. Aldous, 9/4/96.

MUTAGENICITY, Reports not acceptable: not guideline studies.

[no document or record number] Taets, C., S. Aref, and A. L. Rayburn, "The clastogenic potential of triazine herbicide combinations found in potable water supplies," study conducted at Department of Crop Sciences, Univ. of Illinois, Urbana, published April 1998 in **Environmental Health Perspectives** 106, 197-201. Atrazine, simazine, and cyanazine were tested individually and in combination in CHO cells for 48 hr at the M.C. L. and at the highest level found in Illinois water supplies (3 and 18 ppb for atrazine, respectively). Evaluations of nuclei were done by lysing cells, staining with propidium iodide, followed by flow cytometry using fluorescence detection. The parameter of interest was the coefficient of variation (CV) of the G1 peaks. Evaluations of chromosomes involved similar incubations, followed by colcemid treatment prior to staining. Cells were broken and chromosomes separated by forcing through a syringe needle. The measured parameter was the CV of the peak of largest chromosome. Atrazine by itself and in most combinations with the other triazines significantly increased the CV of whole nuclei G1 peaks and of the largest chromosome. These results were taken by investigators to indicate clastogenicity, however the meaning of the results is unclear, since this is not a validated assay design and the measured parameter cannot unambiguously be attributed to clastogenicity. Aldous, 5/28/98.

[no document or record number] Biradar, D. P., and A. L. Rayburn, "Chromosomal damage induced by herbicide contamination at concentrations observed in public water supplies," study conducted at Department of Crop Sciences, Univ. of Illinois, Urbana, published in **J. Environ. Qual.** 24:1222-1225 (1995). No essential information is provided here above what is presented in the previous entry from Dr. Rayburn's laboratory, except that investigators show that a known clastogen [cytosine β -D-arabinofuranoside ("Ara-C")] increases the CV of the frequency distribution of the largest chromosome of CHO cells, as does atrazine, suggesting that by association, atrazine may be a clastogen. In addition, Ara-C and atrazine significantly reduced the percentage of total chromosomes observed in the peak corresponding to the largest chromosome, however this was a less sensitive measure than the change in the CV frequency distribution of the largest peak. No worksheet. Aldous, 5/29/98.

[no document or record number] Biradar, D. P., and A. L. Rayburn, "Flow cytogenetic analysis of whole cell clastogenicity of herbicides found in groundwater," **Arch. Environ. Contam.**

Toxicol. 28, 13-17 (1995). This is the earliest of 3 similar publications (see above). Useful additional information is that the positive control, "Ara-C," causes marked "smearing" of the AG1" peak when tested at 0.3 μM concentrations, however at 3.0 μM , the G1 peak disappeared and a new well-defined peak appeared, suggesting that Ara-C had effectively blocked the cell cycle. In addition, when atrazine was incubated for only 15 minutes in this system at concentrations shown to elicit an increase in the CV of the frequency distribution of the G1 peak following the standard 48 hr incubation, the histogram generated by the cytometer appeared normal, suggesting that the mere presence of atrazine in the nucleus at time of assay did not alter the histogram. No worksheet. Aldous, 5/29/98.

061 040000 (Mutation Research, 1971) One-paragraph summary. No adverse effects indicated. Insufficient information to make any conclusions. J. Schreider, 4/18/86.

053 016896 Exact duplicate of 061 040000.

061 040001 (J. of Toxicology & Environ. Health, 1977) Summary of miscellaneous studies with *Drosophila*. **Possible adverse effect noted:** reductions in egg hatches, losses of x or y chromosomes. J. Schreider, 4/18/86.

053 016894 Exact duplicate of 061 040001.

061 040003 (Mutation Research, 1980) Half-page summary. Miscellaneous assay systems discussed. Insufficient information for meaningful conclusions. No adverse effects noted. J. Schreider, 4/18/86

053 016894 Exact duplicate of 061 040003.

061 040004 (Abstract from Mutagen Society, 1981) Summary of discussion of different protocols for cytogenetic studies. No adverse effects reported. J. Schreider, 4/18/86

053 016892 Exact duplicate of 061 040004.

220-355 137848 Brusick, D. J., "An assessment of the genetic toxicity of atrazine: Relevance to human health and environmental effects," [not a laboratory study, but an evaluation of the overall mutagenicity study database]. Analysis was by a computer-assisted weight of evidence approach, assessing the composite results of reviewed data under various study designs. Conclusion by Dr. Brusick was that "an adequate case for classifying atrazine as a genotoxic hazard (or genotoxic carcinogen) to humans has not been made." No worksheet (not an independent study), C. Aldous, 7/25/95.

METABOLISM (and related dispositional studies)

Human and Primate Studies:

220-468 151496 Hui, X., R. Wester, H. I. Maibach, B. Simoneaux, and C. Breckenridge, "Disposition of atrazine in Rhesus monkey following oral administration," Surge Laboratory, Dept. of Dermatology, UCSF, San Francisco, CA and Biochemistry Department, Ciba Crop Protection, Greensboro, NC, 10/30/96. UCSF Study 96SU01, Ciba Study No. 306-96. Nine female Rhesus monkeys were assigned to this study. There were 3 dose groups, each dosed once

orally with either 1, 10, or 100 mg atrazine. Weights of low and high dose monkeys were 9.1 to 9.5 kg, whereas weights of mid-dose monkeys ranged from 5.0 to 6.8 kg. Four monkeys were assigned to each group (three of the monkeys dosed with the low dose were later administered the high dose after 25 days). Urine, feces, and whole blood were collected throughout a 168-hr period after dosing, after which animals were returned to the colony. Re-use of three monkeys was not likely to have influenced results. Parent atrazine and several expected metabolites were evaluated [G-30033 (deethylatrazine), G-28279 (deisopropylatrazine), and G-28273 (diaminochlorotriazine or DACT)] in urine, but no metabolite analyses were performed in feces. Total recoveries were 91-95% in the three groups. Total urinary chlorotriazines during hours 0-48 accounted for 23.0%, 32.5%, and 28.4% of administered dose in the low to high dose groups, respectively (no dose-response in the study dose range). Metabolites G-30033, G-28279, and G-28273 accounted for 9.60%, 1.25%, and 17.05% of administered dose (mean values derived from all 3 dose groups), respectively. Parent atrazine was normally not detectable in urine. Urinary triazine mercapturates constituted only about 0.1 to 0.4% of administered dose. Primary purpose of the study was to determine what analytes could serve as biomarkers of atrazine exposure, hence results confirmed that assays for the de-alkylated chlorotriazines should be useful for that purpose. This study provided valuable information about the fate of atrazine in a primate. Approximately 66% of the administered dose was absorbed, as indicated by presence of residues in urine plus cage wash. Over 50% of residues recoverable in urine were composed of de-alkylated atrazine products, with DACT being the most abundant. Limitations of the study were that nearly one-half of the urinary metabolites were not characterized, and that no fecal metabolite characterization was undertaken. Valuable supplementary data. Aldous, 1/23/08.

220-467 151495 Hui, X. *et al.*, "Disposition of atrazine in Rhesus monkey following intravenous administration," (collaborative effort with participants at UCSF, UCD, and Ciba Crop Protection), Sept. 3, 1996. Several reports comprise this DPR record, most of which deal with monkey metabolism, however some human volunteer data are included. The UCSF analyses of disposition data from 4 monkeys concluded that 62.5% of administered dose was excreted within 24 hours, with a 2-compartment model yielding blood clearance $T_{1/2}$ values of 1.5 and 17.7 hours. By 7 days, 81-85% of administered dose was found in urine and 11.7% in feces (hence nearly complete accountability of label by these two routes: pp. 6-7). Urine studies found no atrazine, and incomplete characterization of metabolites. Identified metabolites (by TLC separation) were 10.7% de-ethyl atrazine, 13.7% de-isopropyl atrazine, 4.4% atrazine mercapturate, and 5.6% di-dealkylated mercapturate. In the TLC system used for this study, highly polar metabolites remained near to the origin (i.e. "Unknown #1), and comprised most of the recovered label from urine. This was particularly evident during the first day after exposure (see tables on pp. 100 ff.). Investigators sought to validate an ELISA method for assay of atrazine mercapturic acid as an indicator of exposure. A problem arose in that substantial cross-reactivity occurred with mercapturic acid of simazine (a congener of atrazine), but also with parent herbicides simazine and atrazine, as well as simazine thiopropionic acid (p. 108). Five male human volunteers/group were administered U-ring- ^{14}C -atrazine at low or high dose single topical exposures (0.1667 or 1.9751 mg/person). Skin was washed at 24 hours. Urine was evaluated for 1 week (pp. 231-232). An average estimate of 5.03% of administered dose was excreted in urine at 0.1667 mg/person, and 1.11% of dose was excreted at 1.9751 mg/person. Investigators stated that feces were also examined (pp. 231-232), however no information on fecal label excretion was mentioned in summary statements nor tables, except for a general statement (p. 5): "Approximately 90% of the absorbed dose appeared in the urine and 10% in the feces. Detailed quantification of metabolic profile of atrazine in man was not possible in this study because of the restricted dose administered and absorbed. However, dealkylated

metabolites of atrazine and a limited amounts [sic] of the mercapturate of atrazine were found.” Aldous, no DPR worksheet, 7/25/07.

220-113 069692 Davidson, W. F., “Metabolism and kinetics of atrazine in man,” Project 101947 (unpublished study from Bowman Gray School of Medicine, U.S. EPA gives date as 1988). Six human subjects (sex not specified) each consumed a single dose of 0.1 mg/kg unlabeled atrazine. Urine collection and periodic blood sampling were conducted for 1 week for atrazine plus three metabolites: its two N-dealkylation products [removal of either the ethyl group (G-30033), or the isopropyl group (G-28279), or the di-dealkylation product (2,4,-diamino-6-chloro-s-triazine) designated G-28273. Atrazine and G-28279 were observed below quantitation limits in whole blood. Estimated elimination of these three metabolites in urine were 5.4% of administered dose for G-30033, 2.4% of dose as G-28279, and 7.8% of dose for the di-dealkylated G-28273. G-30033 was quickly cleared from whole blood, with a $T_{1/2}$ of 2.8 hr. G-28273 had a $T_{1/2}$ of 17.8 hr. This study has some value in that it accounts for about 15% of an administered dose of atrazine, however there are evidently other large metabolic pathways not identified. Aldous, 7/24/07 (no worksheet).

220-0357 137850 This appears to be the same report as 220-113 069692, above.

[Publication, without DPR Document No./Record No.] Barr, D. B., P. Panuwet, J. V. Nguyen, S. Udunka, and L. L. Needham, “Assessing exposure to atrazine and its metabolites using biomonitoring,” **Environmental Health Perspectives** **115** (10):1474-1478. This article was not directed to DPR Data Review Group for examination, however the study appears to account for the principal metabolites in humans exposed occupationally or incidentally to atrazine (i.e., no intentional dosing). Investigators determined that DACT was the most abundant metabolite (48-77% of residues), with G-30033 (desethylatrazine: 15-43% of residues), and G-28279 (desisopropylatrazine: 2-6% of residues). Atrazine mercapturate was found at 2-12% of observed residues. Atrazine, hydroxydesethylatrazine, and hydroxyatrazine were occasionally found as very minor residues. Investigators observed that there was great variability between metabolite profiles between individual persons, concluded that no single metabolite should be singled out as an efficient estimator of exposure. Aldous, 11/13/07 (no worksheet).

Rat, Oral Route:

220-0102 062750 “Disposition of Atrazine in the Rat (General Metabolism),” (Ciba-Geigy Corporation, 10/23/87). ^{14}C -Atrazine (>98% purity) and unlabeled atrazine technical (purity not stated) was administered (gastric intubation) to three groups of 5 CR CD rats/sex/dose and 2/sex/dose were untreated controls. Group 1 was given a single oral dose at 1.0 mg/kg. Group 2 was given a single oral dose at 100 mg/kg and Group 3 was subchronically treated for 14 days with unlabeled atrazine given by gavage as a single daily dose at 1.0 mg/kg. On day 15, a single oral dose of ^{14}C -atrazine was administered at 1.0 mg/kg. No significant differences were observed between mean % recoveries of dosage groups or between mean % recoveries for male and female animals within dosage groups. Urine was the primary route of excretion (about 74% of administered dose). About 19% of dose was found in feces. In tissues, the highest concentration of radioactivity was associated with RBC's. The whole body half-life of elimination was determined as 31.3 ± 2.8 hours and elimination was from two body compartments by first order process. No adverse effects indicated. UNACCEPTABLE, (no analysis of atrazine technical, or dosing material; need to state whether high dose caused toxic or pharmacologic signs; intravenous route of dosing was not utilized). Upgradeable. M. Silva,

5/20/88, 1-liner amended on 7/24/07 by Aldous at request of DPR Risk Assessment Group, without supplementary worksheet.

220-0113 069697 This record is nearly identical to the record reviewed above (220-102 062750).

102 062287 "Study of ^{14}C -Atrazine Dose/Response Relationship in the Rat (General Metabolism)," (SRI International and Agrisearch Incorporated, 10/23/87). ^{14}C -Atrazine (purity = 97.9%), in combination with cold atrazine (purity = 98.8%) was administered by gastric intubation to Sprague-Dawley CD female rats at 0 (vehicle = corn starch/polysorbate 80), 1, 3, 7, 10, 50 and 100 mg/kg daily for 10 days (2 rats/group). One rat/group was sacrificed 3 hours after the tenth dose and the remaining animals were sacrificed 72 hours after the tenth dose. Recovery averaged 89.2% (3 hour sacrifice after tenth dose), and 94.2% (72 hour sacrifice after tenth dose). The primary route of excretion was urine. A steady-state RBC concentration was estimated to be achieved only after 30 days of daily dosing. Tissue concentrations at 3 hours after the tenth dose were linearly related to plasma concentrations obtained at the same time point. Tissue concentrations at 72 hours after the tenth dose were 27% (significant) lower. A longer residence time for a portion of tissue ^{14}C -radioactivity than plasma radioactivity was indicated. Subchronic administration does not alter the first-order kinetics pattern of excretion observed for a single dose of atrazine. No adverse effect indicated. This study is supplemental to 102 62750. M. Silva, 5/20/88.

220-0113 069695 This record contains a major portion of the record reviewed above (220-102 062287).

102 062647 "A Summary of the Disposition, Kinetics and Metabolism of Atrazine in the Rat," (Ciba-Geigy Corporation, 11/17/87). ^{14}C -Atrazine (purity not stated), was administered in a single oral dose to Sprague Dawley CD rats (5/sex/group) at 1.0 mg/kg, 100 mg/kg and 1.0 mg/kg following a 14 day period of dosing with non-labeled material at the same rate. Elimination of radiolabel in urine and feces was monitored over a 7-day period after which the animals were sacrificed. A second test was run where female rats (2/group) were dosed daily for 10 days with ^{14}C -atrazine at 0 (vehicle = corn starch/polysorbate-80), 1, 3, 7, 10, 50 and 100 mg/kg, then sacrificed. In a third test, 5 females were dosed with 100 mg/kg to obtain metabolites for isolation and identification. Urine was the preferred form of elimination (first order, two compartment open system with whole body half-life = 31.3 hrs). [See findings of Record No. 064323, below, for discussion of metabolites observed]. No adverse effect indicated. This study is supplementary to 102 62750. M. Silva, 5/20/88.

220-0113 069694 This record contains a major portion of the record reviewed above (220-102 062647).

102 064323 & 064324 "Characterization and Identification of Atrazine Metabolites From Rat Urine (General Metabolism)," (Ciba-Geigy Corporation, 11/17/87). ^{14}C -Atrazine (> 97% pure) was administered (vehicle = 1% carboxymethyl cellulose and Hi Sil 233 silica gel) by gavage in a single dose to 5 female Sprague-Dawley rats at 100 mg/kg. Urine and feces were collected daily, and rats were sacrificed at 72 hr, with examination of tissues for radiolabel. Several metabolites were characterized. A mean total of 47.37% of the dose was excreted in the urine (about 25%, 20%, and 3% on days 1, 2, and 3, respectively), 49.26% in the feces (about 24%, 17%, and 8% on days 1, 2, and 3, respectively), 1.37% in the blood, and the rest in other tissues. Recovery averaged 103.78% with 93.5% of the administered dose eliminated within 72 hours.

Major products as percent of urinary label included 2,4-diamino-6-hydroxytriazine (54%); a fraction which included hydroxyatrazine and its two N-dealkylation products (removal of either the ethyl or isopropyl group), (8.5%); two unresolved hydroxyatrazine products, each with one of the alkyl carbons oxidized to a carboxylic acid (5.6%); one more substantially metabolized product, 2-hydroxy-4-amino-6-N-(hydroxyglycyl)-s-triazine (5.5%). Investigators noted that it is probable that the hydroxylation of the carbon bearing the chlorine was an artifact of the sample preparation: indicating that the primary metabolites were products of N-dealkylation, with limited oxidation of the N-alkyl groups. Some metabolism at the chlorine-bearing carbon was, however, substantiated by preparing a urine sample with minimal manipulation and analyzing by GLC, which indicated 2,4-diamino-6-methylthio-s-triazine (by comparison with retention times of an authentic standard). Thus conjugation by glutathione may be at least a minor route in rats. Report 102 064324 "Formulation and Analysis of ¹⁴C-CGA-169374 Dose Suspensions for Rat," (Ciba-Geigy, Greensboro, NC 2/10/87) contained an analysis of dosing material. These data are supplementary to report 102 62750. M. Silva, 5/20/88, amended on 2/9/07 by Aldous.

220-0113 069696 This record is nearly identical to the record reviewed above (220-102 064323).

103 064326 "Metabolism of ¹⁴C-Atrazine in Orally Dosed Rats," ¹⁴C-Atrazine, (radioactive purity, 97.5%) was administered orally to male Harlan Sprague-Dawley rats for 7 days at 0.4 and 4.0 mg/kg. Three rats/time point were sacrificed at 5, 7, 9, 10, 14 and 18 days after dosing. The peak levels of radioactivity in RBC, liver and kidney were reached at Day 8 for 4.0 mg/kg treated animals and Day 10 for 0.4 mg/kg treated animals. There were no significant differences observed in excretion patterns between the two dose levels. No adverse effect indicated. This study is supplementary to 102 062750. M. Silva, 5/20/88.

220-104 064329 "Metabolism of 2-chloro-4,6-bis(isopropylamino)-s-triazine (Propazine) and 2-Methyl-4,6-bis(isopropylamino)-s-triazine (Prometone) in the Rat. Balance Study and Urinary Metabolite Separation," (USDA, ARS, 1967). The excretion of Propazine-¹⁴C and Prometone-¹⁴C (98-100% pure) labeled in the triazine ring was determined following administration of a single oral dose (41-56 mg/kg) to Sprague-Dawley male rats (5/group). Prometone-¹⁴C activity was quantitatively recovered in the urine and feces within 72 hours after treatment. Propazine-¹⁴C activity was excreted more slowly with tissue residues remaining 12 days after treatment. No activity could be detected in the expired CO₂ with either ring-labeled compound. Ion exchange chromatography of the urine revealed at least 11 metabolites from Prometone and 18 from Propazine. M. Silva, 5/2/88. NOTE: the metabolites were not analyzed in this study. One segment of this study involved dosing with ¹⁴C-label in the isopropyl groups of propazine [the analog of atrazine differing in having 2 isopropyl groups instead of one isopropyl and one ethyl group]. When the disposition this alkyl label was evaluated, investigators noted that about 50% of radioactivity was observed in exhaled CO₂, with the balance of the label being found primarily in urine and feces, plus smaller amounts in the tissues. The presence of exhaled CO₂ only in the alkyl-label tests indicates that de-alkylation is a major metabolic process, and that ring cleavage is not observed. (Additional notes were prepared by Aldous in support of Risk Assessment Group, without supplementary worksheet, on 7/24/07).

104 064330 "The Transformation of Triazine Herbicides in Animals" (Max von Pettenkofer-Institut, 1967). Male albino rats and male rabbits (number of animals was not specified) were treated by esophageal probe (number of treatments not specified) with simazine, atrazine, propazine, prometone or prometryn at 50-200 mg/animal (rats) or 600-1000 mg/animal

(rabbits). Actual number of groups treated was not indicated. The urine was collected during the first 72 hours after feeding. A number of metabolites were isolated and identified. All metabolites retained the triazine ring intact. Dealkylation was a common metabolic pathway. M. Silva, 5/2/88. [Investigators did not provide quantitative values, but noted also that no observed metabolites displayed cleavage of the chlorine group - Aldous, 7/24/07].

Rat, Dermal Route:

103 064325 "Dermal Absorption of ^{14}C -Atrazine in the Rat (General Metabolism)," (Ciba-Geigy Corporation, 11/11/87). Atrazine (radioactive purity = 99.5% for low & mid doses, 99% for the high dose) labeled with ^{14}C and then dermally applied to male CR Sprague-Dawley rats (4/group) at 0.1, 1.0, or 10.0 mg/rat using 4L formulation. Another group used atrazine (10 mg/rat) in an 80W formulation. The application site was covered with a non-occlusive bandage. Rats treated with atrazine formulated with 4L were sacrificed at 2, 4, 10 or 24 hours after treatment. Rats treated with atrazine formulated with 80W were sacrificed at 10 hours. There was no difference in atrazine absorption, excretion or the amount left unabsorbed at 10 hours using 4L versus 80W. No adverse effects indicated. This study is supplementary to 102 62750. M. Silva, 5/20/88. NOTE: There was no systematic difference in amount absorbed at a given dose at either of the four time periods. Using the 10-hr time period for comparison across dose levels, total absorption for 0.1, 1, or 10 mg/rat was 27%, 22%, and 11%. This reduced absorption at 10 mg/rat at 10 hr is typical of other time periods. Typically over 90% of label considered to be "absorbed" was obtained from the dissected skin after detergent washing and rinsing had removed superficial material. At a maximum, 2.5% of administered label was obtained in urine and 0.33% in feces (24-hr sacrifice group of 0.1 mg/rat treatment). Thus systemic dermal absorption in rats was quite limited compared to oral exposure. (Additional notes were prepared by Aldous in support of Risk Assessment Group, without supplementary worksheet, on 7/24/07).

220-0113 069698 This record contains one of three reports comprising the record reviewed above (220-103 064325).

103 064327 "Dermal Absorption of ^{14}C -Atrazine by Rats," (Ciba-Geigy Corporation, 5/16/83). ^{14}C -Atrazine dissolved in ethanol was applied dermally at 0.25 and 2.5 mg/kg to Harlan Sprague-Dawley rats for 2, 4, 8, 24, and 48 hours (4 rats/sex/dose at each time point), then sacrificed. Atrazine had low solubility at 2.5 mg/kg and flaked off the skin, therefore, only data from the low dose was used in determining half-life skin values. Absorption of total radioactivity by the skin did not vary significantly by sex nor did excretion in urine and feces. Tissue levels of radioactivity did not plateau by 72 hours at the low dose. Most radioactivity was excreted in the form of ^{14}C -atrazine at both dose levels. No adverse effect indicated. This study is supplementary to 102 62750. M. Silva, 5/20/88. NOTE: This study used ethanol as solvent, and reported about 70% absorption as of 72 hr after dosing at 0.25 mg/kg. This is a stark contrast to the very low dermal absorption reported with an aqueous suspension in study 220-103 064325 (above). Urinary excretion averaged 35%, and fecal excretion averaged 11% of administered dose. About 1% of administered dose was found in dissolved skin, about 1% in blood, and about 18% in sampled tissues plus carcass. It is likely that the much lower absorption reported for the use formulation in 220-103 064325 would be more predictive of plausible exposure than the high absorption reported in the present study. (Additional notes were prepared by Aldous in support of Risk Assessment Group, without supplementary worksheet, on 7/24/07).

103 064328 “Excretion Rate of ^{14}C -Atrazine From Dermally Dosed Rats,” (Ciba-Geigy Corporation, 10/20/88). ^{14}C -Atrazine dissolved in tetrahydrofuran with a specific activity of 17.2 mCi/mg was dermally applied at 0.025, 0.25, 2.5 and 5.0 mg/kg to 2 female Harlan Sprague-Dawley rats/group for 144 hours. Urine and feces were collected at 24-hour intervals. An increase in percent of dose excreted was observed as the dosage level increased. The amount of dose remaining on skin residues was observed to decrease as dosage increased. The main route of excretion was in the urine. No adverse effect indicated. This study is supplementary to 102 62750. M. Silva, 5/20/88. NOTE: Urinary excretion was 50-59% of administered dose in the three higher dose groups, and fecal excretion was 20-21% of dose in these groups, indicating that there was no apparent saturation of absorption in this range. The lowest dose rats had somewhat lower urinary and fecal excretion, possibly a function of normal variability. This study appears to reflect much higher absorption than was reported for the aqueous suspension tested in 220-103 064325, apparently greatly influenced by the organic solvent vehicle. (Additional notes were prepared by Aldous in support of Risk Assessment Group, without supplementary worksheet, on 7/24/07).

220-0113 069699 This record contains an earlier version of a record reviewed above (220-103 064328).

Rat, *in Vitro*:

104 064331 “In Vitro Metabolism of Atrazine by Rat Liver,” (Ciba-Geigy Ltd., 1973). Male RAI rat livers were homogenized to make a 10,800 g fraction, and 100,000 g supernatant and microsomal fractions. The metabolism of atrazine and 6 possible metabolites by rat liver subcellular fractions was studied in vitro. The dealkylation reaction predominated over the conjugation reaction with glutathione; the isopropyl group was more easily dealkylated than the ethyl group. With the compounds investigated, the reactions involved dealkylation in the microsomal fraction and conjugation with glutathione in the soluble fraction. All of the chloro- s -triazines were able to form conjugates with glutathione. No evidence for the dechlorination of the chloro- s -triazines to hydroxy- s -triazines was observed in vitro. M. Silva, 5/2/88.

104 064332 “Metabolism of Mercapturic Acid-pathway Metabolites of 2-Chloro-isopropylacetanilide (Propachlor) by Gastrointestinal Bacteria,” (Metabolism and Radiation Research Lab, USDA, 1983). The study was published in Xenobiotica, Vol. 13, No. 2, 115-126, 1983. There was no worksheet done on this supplementary information. M. Silva, 5/23/88.

213-0086 090525 Knaak, J. B. and S. H. Caballa, “The *in vitro* metabolism of ^{14}C -atrazine and derivatives by rat and sheep liver under tissue culture conditions,” Ciba-Geigy Corporation, Ardsley, NY, May 4, 1973. This supplementary study used “liver cubes” in medium to evaluate *in vitro* metabolism of atrazine and of its dealkylated metabolites. Investigators determined that atrazine was partially dealkylated under these conditions, and that atrazine and its metabolites reacted to a small extent with glutathione to form conjugation products. Supplementary data, not suitable for DPR worksheet. Aldous, 11/15/07. (Record submitted in support of simazine).

Commentaries, often addressing several species:

220-356 137849 Simoneaux, B. J., “Nature of atrazine and simazine metabolism in animals and plants,” a Ciba Crop Protection review document, March 9, 1995. This article is an overview of many documents on the fate of atrazine and simazine in rodents, farm animals, humans, and in several fruit, cereal, and vegetable crops. Several documents on human disposition of triazines

appear to be in conflict, perhaps reflecting different techniques, routes, or purposes of evaluation. Ikonin *et al.* (ref. 10, cited on p. 22) determined that humans exposed occupationally to atrazine dusts produce “near equal amounts of 2-chloro-4-amino-6-(ethylamino)-s-triazine and 2-chloro-4,6-diamino-s-triazine in their urine samples.” It is not clear whether this pair of metabolites was considered to represent the bulk of metabolites present. Catenacci *et al.* (ref. 12, cited on p. 21) stated regarding a similar worker exposure that “total urinary atrazine excretion accounted for 1-2% of the external dose. The spectrum of urinary chlorotriazine metabolites comprised bi-dealkylated (80%), deisopropylated (10%), deethylated (8%), and unmodified atrazine (2%).” Lucas *et al.* (ref. 13, cited on p. 21) examined urine of field applicators of atrazine using an ELISA technique, seeking an effective biomarker for atrazine exposure. They identified a mercapturic acid conjugate “in concentrations at least 10 times greater than any of the N-dealkylated products or the parent compound.” Subsequent cited articles focused in large part on urinary excretion of atrazine N-dealkylation products. In the absence of a report of an occupational exposure study which provides full accountability for urinary residue samples, the true extent of atrazine metabolism, including N-dealkylation and extent of oxidation or conjugation and subsequent modifications of substituents of the chlorine-bearing carbon, will remain unclear. Aldous, no worksheet (not a reviewable study), 2/13/07.

220-0104 230286 Hamboeck, H., R. W. Fischer, E. E. Di Iorio, and K. H. Winterhalter, “The binding of s-triazine metabolites to rodent hemoglobins appears irrelevant to other species,” *Molecular Pharmacology* 20:579-584, 1981. Many tests were performed with simetryn (an analog of simazine, differing in that the chlorine is replaced by an -SCH₃ substituent). When C₁₄-labeled simetryn was added to rat whole blood, very little label was found in the RBC's. In contrast, *in vivo* administration of simetryn led to about 95% of label in blood being found in RBC's. A key metabolite of simetryn incubated with rat liver microsomes was simetryn sulfoxide (21.5% of recovered label). This sulfoxide, when incubated with rat blood, became concentrated in RBC's, and the label could not be removed by dialysis. This indicates covalent binding. Similar binding to RBC macromolecules was observed with blood from guinea pigs and chickens, but not with blood from man, dog, pig, cow, or sheep. Labeled simetryn sulfoxide reacted with GSH yielded a modified cysteine upon digestion: this product was evidently the same as that which was obtained upon digestion of hemolysates after incubation with simetryn sulfoxide. Also, pre-treatment preparations with iodoacetamide (which binds tenaciously to -SH groups) inhibited the binding of simetryn sulfoxide. Evaluations of hemoglobin fractions following treatment of blood with C₁₄-labeled simetryn sulfoxide found nearly all label in the β-chain (apparently one molecule per tetramer). Subsequent detailed studies of the binding location on the β-chain indicated that simetryn sulfoxide binding was specific to the β-125 residue in rodents, for which there does not appear to be an amenable binding site in the analogous region of humans nor of most species evaluated. Subsequent studies of *in vitro* blood binding were performed with atrazine and ametryn (simetryn analog, with the N-alkyl groups of atrazine instead simazine), and with the sulfoxides of these two molecules. Only the sulfoxides retained significant label after dialysis, and strong binding in these cases were limited to rodents (binding being particularly weak in humans). These studies indicate that the appreciable label retention of triazines previously observed in rats is essentially rodent-specific and requires metabolism to sulfoxides for covalent binding. Useful data presented in a major journal, but not amenable to DPR review of individual data. No DPR worksheet. Aldous, 2/14/07.

220-113 069693 Simoneaux, B. and B. Thede, “Comparative metabolism of atrazine by mammalian tissue cultures: preliminary report,” (Ciba-Geigy, Greensboro, NC), 5/16/88. This report evaluated relative amounts of mono-dealkylated atrazine metabolites, G-28279 and G-30033 (see above paragraph) following incubation with cultures of hepatocytes from Fischer rat,

goat, and human. Under test conditions, human hepatocytes produced more of the de-ethylated metabolite (G-30033) than of the de-isopropylated metabolite (G-28279). There was one other appreciable unidentified peak associated with human hepatocytes that was intermediate in quantity between the above characterized metabolites. Useful supplementary data. Aldous, no worksheet, 7/24/07.

NOTE: An assessment by Silva, 5/88, of metabolism studies reviewed as of that time found sufficient information to address metabolism data requirements.

NEUROTOXICITY, HEN

Not required at this time.

GENERAL REVIEW DOCUMENTS ON ATRAZINE:

220-336 137769 "Atrazine/Simazine Response to the United States Environmental Protection Agency's Position Document 1: Initiation of Special Review," PD-1 was dated 11/23/94. The primary Ciba response, entitled "Evaluation of the carcinogenic potential of atrazine and the relevance to human risk assessment" was dated 3/22/95. This document did not contain unique SB-950 data. Principal issues were the same as discussed in 220-222 128816 (Eldridge *et al.*, 1993), found in the Combined, Rat section, above. No SB-950 review is appropriate at this time. Aldous, 7/30/96.

220-337 137772 [A parallel response to Record No. 137769, above, except relating to simazine]. No SB-950 review is appropriate at this time. Aldous, 7/30/96.

PROPOSED PROTOCOLS FOR UPCOMING STUDIES:

220-394 137917 Breckenridge, C. *et al.*, (proposals to undertake several studies in female Sprague-Dawley rats to evaluate luteinizing hormone surge as it relates to estrous cycling). These studies were completed or well underway as of 3/31/98.

220-424 146006 Proposed protocol for the "fourth study" mentioned in the preceding paragraph. The interim report for this study (CHV Study No. 2386-108) has since been reported as Record No. 151475. No DPR worksheet is necessary. Aldous, 9/5/96, updated 3/19/98.

STUDIES OF ATRAZINE METABOLITES, CONTAMINANTS, OR ANALOGS:

Chronic, Oncogenicity, Or Subchronic Rat (Atrazine-Related Compounds):

220-370 137863 Tennant, M. K. *et al.*, "Ultrastructural changes in rat hypothalamic arcuate nucleus following long-term diaminochlorotriazine feeding," *Steroid Biochem.* 12:21-26 (1993).

Female Sprague-Dawley rats were treated for 12 or 28 weeks with 1000 ppm DACT. EM evaluation of arcuate nucleus sections was performed, in comparison to controls. There was an increase ($p < 0.05$) in astrocyte granule density in treated rats after 28 weeks of treatment only (based on 3 treated rats at that time period). No other treatment-related effects were observed. These granules increase during aging and rate of appearance is influenced by factors including levels of several circulating hormones. Investigators proposed that circumstances associated with prolonged estrus in treated rats may be related to the hypothalamic changes. Aldous, 7/31/95 (only highly reduced data provided, hence no DPR worksheet).

220-332 134724 Chow, E. and Emeigh Hart, S.G., "2-Year dietary chronic toxicity/ oncogenicity study with G-34048 in rats," Ciba-Geigy Corporation, Farmington, CT., 1/27/95. Report # F00125. G-34048 Technical (hydroxyatrazine), purity 97.1%, was administered in diet to 70 to 80 CD7 rats per sex per group at 0, 10, 25, 200, or 400 ppm. Sixty/sex/group were designated for a 2-yr study, other rats were sacrificed at 12 months. NOEL = 25 ppm (progressive nephropathy in females; dilatation with crystalline deposits, particularly in collecting ducts and renal pelves, interstitial papillary fibrosis). High dose rats had an accelerated onset of severe progressive nephropathy, with associated widespread mineralization of tissues, secondary parathyroid hyperplasia, and associated changes in bone (fibrous osteodystrophy), heart (progressive cardiomyopathy), and testes (degeneration or atrophy). These findings showed a sharp dose-response relationship, were limited to relatively high dose levels, and did not represent the active ingredient. **Acceptable** as an ancillary study. (H. Green and C. Aldous, 10/3/96).

220-417 140111 Preliminary U.S. EPA DER of 220-332 134724, above. No DPR worksheet of this preliminary review is necessary. Aldous, 9/5/96.

220-333 134857 This is a 12/22/94 "potential adverse effects" disclosure originally sent to U.S. EPA regarding the nephrotoxicity evaluated in Record No. 134724, above. (No DPR "review" of this disclosure). Aldous, 8/30/96.

220-180 120943 1-year interim report for Record No. 134724, above. Briefly reviewed by Aldous, 10/7/93.

220-314 131713 June 1994 interim report for Record No. 134724, above. No review necessary, since final report is available.

220-216 128786 Rudzki, M. W., G. C. McCormick and A. T. Arthur, "Hydroxyatrazine: 90-day oral toxicity study in rats," Ciba-Geigy Corp., Summit, NJ, 10/25/89. Dose range-finding study for the chronic hydroxyatrazine study (Record No. 134724, above). Main finding was nephrosis at 300 to 600 ppm, with associated changes in serum chemistry, urine volume, and urine solute concentrations. Apparent NOEL = 100 ppm. No worksheet (2-year study has been reviewed, with lower NOEL). No adverse effects. No further information required. Aldous, 5/11/98.

220-215 128785 Pettersen, J. C., A. D. Richter, and P. A. Gilles, "Diaminochlorotriazine (G-28273): 90-day oral toxicity study in rats," Ciba-Geigy Corporation, Farmington, CT, 11/5/91, Laboratory Study # F-00006. Test article is termed "DACT" in this review. Crl:CD7(SD)BR rats, 15/sex/group, were dosed with 0, 10, 100, 250, or 500 ppm DACT (98.2%) in diet in a guideline study. In addition, estrous cycling was evaluated by daily vaginal smears (the main evaluation periods involving all rats encompassed days 42-56 and 70-85). Shortly before

termination, blood samples were taken for hormonal evaluations, with an attempt to bleed during proestrus at a fixed time of day to optimize sensitivity and standardize diurnal variables (with analysis by an outside specialist: not under GLP oversight). Apparent NOEL was 10 ppm, based on increased numbers of “variable” or “indeterminate” length estrous cycles (< 4 days or > 5 days) at 100 ppm and above during days 42-56 (a finding more common in higher treatment groups at both of the main evaluation periods). Despite considerable variability in estrous status evaluations, incidence of “persistent estrus” was elevated at 250 and 500 ppm compared to other groups. No hormonal effects could be detected in this study, which was complicated because limited numbers of rats in the higher two dosage groups were in proestrus at blood sampling time, hence few usable sampling units. Other findings were reduced body weights at 500 ppm (significant only in males), and a small but statistically significant reduction of serum calcium, of unlikely physiological importance. This is an **acceptable** ancillary study. Aldous, 6/1/98.

220-213 128783 Schneider, M., “G-28279 Technical: 3-month oral toxicity study in rats (administration in food),” CCBA-Geigy Limited, Stein, Switzerland, 5/8/92, Test #: 901261. Ten Tif: RAIf (SPF) rats/sex/group were dosed with 0, 10, 50, or 500 ppm atrazine metabolite “AG-28279” [deisopropylatrazine], batch FL-901747, 96.7% purity for 3 months in a standard subchronic study design. NOEL = 10 ppm [increased extent of splenic extramedullary hematopoiesis (EMH) in females]. Common findings at 500 ppm included body weight decrements (significant, $p < 0.01$, in females), marked increase in water consumption (without associated pathology), pituitary cell hypertrophy with associated thyroid follicular epithelium hypertrophy and fatty change in adrenal cortex in males, liver EMH and increased splenic EMH in females. Acceptable ancillary study, with no adverse effects. Aldous, 5/8/98.

220-213 128784 Gerspach, R., “G-30033 Technical: 3-month oral toxicity study in rats (administration in food),” Test No. 901264, 10/22/91. G-30033 (deethylatrazine) elicited minor body weight decrements and slight alterations in hematology and serum chemistry at 500 ppm, so that the NOEL was the next lower dose of 50 ppm. No worksheet. No adverse effects. **No changes** in splenic extramedullary hematopoiesis at any dose level, in contrast to the deisopropylatrazine subchronic report in Record No. 128783, above. Aldous, 5/11/98.

Chronic or Subchronic, Dog (Atrazine-Related Compounds):

097 060319 “Atrazine: Potential Adverse Effect Finding. Dog Chronic Feeding Study (Metabolite),” (Ciba-Geigy Corporation, Greensboro, NC). A pilot study utilizing the atrazine metabolite, 6-chloro-2,4-diamino-s-triazine (CAS No. 3397-62-4) has been initiated to assess its potential for adverse effects. Dogs were treated with doses of 0, 5, 100 and 1500 ppm in the diet. The study in progress demonstrated a **possible adverse effect**. At the end of week 5, four out of twenty dogs treated at 1500 ppm experienced atrial fibrillation. Two of these four dogs also experienced pre-cordial trill (pronounced atrial fibrillation). Currently (13 weeks into the study), the high dose is being lowered to 1000 ppm and two dogs from the 1500 ppm group are being placed on control diet as a recovery group. NOTE: See final report in 220-132 086117. M. Silva, 11/20/87.

**** 132 086117** “Diaminochlorotriazine, 13/52 Week Oral Toxicity Study in Dogs,” (Agricultural Division, Ciba-Geigy Corporation, Summit NJ, 1/17/90). Diaminochlorotriazine (a metabolite of atrazine) technical (98.7% pure; FL 871423) was administered in the feed to Beagle dogs at 0 (10/sex), 5 (8/sex) and 100 ppm (8/sex) for 52 weeks (Groups 1 - 3). Ten males in another group (Group 4) received 1500 ppm (weeks 1-6) and 750 (weeks 7, 8 and 14-52) with

an intervening period of 0 ppm (weeks 9-13). The 10 females in Group 4 received 1500 ppm (weeks 1-6) and 750 ppm (weeks 7-52). At wk 13, an interim sacrifice was performed and 2 Group 4 Females were placed on 0 ppm for weeks 14-52 (recovery). **Possible adverse effect.** NOEL = 100 ppm (Heart pathology and dysfunction, abnormalities of electrocardiogram; clinical signs (associated with decreased heart function) with accompanying gross and histopathological changes in heart and liver; increased liver, spleen and kidney weight; decreased animal weight gain and food consumption; hematology and clinical chemistry effects; increased mortality). ACCEPTABLE. (M. Silva, 3/21/90).

220-406 138979 Chau, R. Y., G. C. McCormick and A. T. Arthur, "Hydroxyatrazine: 13-week feeding study in dogs," Ciba-Geigy Corporation, Summit NJ, 3/20/90. Lab Study No. 892076. Four beagles/sex were dosed with 0, 15, 150, 1500, or 6000 ppm of hydroxyatrazine (presumably about 97.1% purity, based on other studies) for 13 weeks. Hematology, clinical chemistry, and urinalysis were examined pre-study and at termination. Standard gross and microscopic pathology was undertaken. The primary finding was chronic nephropathy with associated crystalline casts in renal tubules and papillae. All dogs at 1500 to 6000 ppm were affected. Acceptable as an ancillary subchronic study on an environmental contaminant. NOEL = 150 ppm (6 mg/kg/day in both sexes). Aldous, 8/7/96, with minor editing of 1-liner on 2/7/07.

220-211 128781 Thompson, S. S., G. Batastini, and A. T. Arthur, "G-28279 Technical: 13-week feeding study in dogs," Ciba-Geigy Corp., Summit, NJ, 4/22/92. Laboratory Study # MIN 912021. Four beagles/sex/group were dosed in diet with 0, 15, 100, 500, or 1000 ppm G-28279 [deisopropylatrazine] for 14 weeks. The study evaluated parameters typical of a dog subchronic study, plus EKG's measured on days -14, 22, 50, and 87. Apparent NOEL = 100 ppm (3.8 mg/kg/day in both sexes), based on reduced body weight and reduced food consumption, particularly in females; reduced organ weights of heart, testis, epididymides, prostate, and uterus; EKG's showed reduced amplitude of P wave in both sexes. The EKG data were evident from the individual dog data, but the text in the report failed to acknowledge or discuss these changes; offering instead the impression that there no treatment-related EKG changes. Study is **not acceptable**, since the QAU failed to identify this reporting discrepancy. No adverse effects are indicated, considering the comparatively high NOEL. Aldous, 5/20/98, with minor editing of 1-liner on 2/7/07.

220-212 128782 Rudzki, M. W., G. Batastini, and A. T. Arthur, "G-30033 Technical: 13-week feeding study in dogs," Ciba-Geigy Corp., Summit, NJ, 4/16/92. Laboratory Study # MIN 902187. Four beagles/sex/group were dosed in diet with 0, 15, 100, or 1000 ppm G-30033 [deethylatrazine] for 13 weeks. The study evaluated parameters typical of a dog subchronic study, plus EKG's measured on days -21, 27, 50, and 78. Apparent NOEL = 100 ppm (3.71 mg/kg/day in males, and 3.88 mg/kg/day in females) [based on reduced body weight and reduced food consumption; reduced organ weights, particularly of uterus and thymus in high dose females, with atrophic change seen in histopathology in both organs; heart pathology was indicated by angiomatous hyperplasia of the right atrium in one high dose male, paroxysmal atrial fibrillation observed EKG's in one high dose female, and quantitative EKG effects including reduced P wave amplitude, increased duration of P waves, and remarkable tachycardia in both sexes; and a reduction of hematology parameters (RBC count, Hb, and HCT) in both sexes]. The quantitative EKG changes were evident from the individual dog data, but the text in the report failed to acknowledge or discuss these changes; offering instead the impression that there no treatment-related EKG changes other than paroxysmal atrial fibrillation in one high dose dog. Study is **not acceptable**, since the QAU failed to identify this reporting discrepancy.

No adverse effects are indicated, considering the comparatively high NOEL. Aldous, 6/1/98, with minor editing of 1-liner on 2/7/07.

Teratogenicity (Atrazine-Related Compounds):

220-293 129150 Giknis, M. L. A., "Hydroxyatrazine Technical: A teratology (segment II) study in rats," Ciba-Geigy Corporation, Research Department, Pharmaceuticals Division, Summit, NJ, Report # 88099, 2/14/89. Hydroxyatrazine technical, 97.1% purity, was administered by gavage in aqueous suspensions of cornstarch/Tween 80 to pregnant Crl:COBS7 CD(SD)BR rats (26 dams/group at 0, 5, 25, or 125 mg/kg/day on gestation days 6-15). Maternal NOEL = 25 mg/kg/day (2 high-dose females had enlarged, mottled kidneys; also there was slightly reduced food consumption at 125 mg/kg/day during gestation days 8-12). Developmental NOEL = NOAEL = 25 mg/kg/day [slightly delayed ossification at several sites, very slight decrement in fetal weights, and a low incidence of pups with malformations in the abdominal wall (1 gastroschisis and 1 umbilical hernia in separate high dose litters)]. The malformations appear plausibly related to treatment, and constitute a **"possible adverse effect" relating to hydroxyatrazine**. The study is **Acceptable** for characterization of toxicity of hydroxyatrazine. (H. Green and C. Aldous, 10/3/96).

220-223 128818 Historical control malformation and skeletal variation data supporting Record No. 129150. Considered in review of that record.

220-224 128820 Chemical analyses of hydroxyatrazine supporting Record No. 129150. Considered in review of that record.

220-229 128904 Similar analytical report to Record No. 128820, but bearing an earlier date and missing even-numbered pages.

220-225 128821 Marty, J. H. "Developmental Toxicity (Teratogenicity) Study in Rats with G-28279 Technical (Oral Administration)," Ciba-Geigy Limited, Stein, Switzerland, Report # 901262, 1 June 1992. G 28279 technical, 97.4% purity. This is deisopropylatrazine (see cross index at end of Summary of Toxicology Data). Tif: RAI f (SPF) rats, hybrids of RII/1 x RII/2, 24 mated females per group, received 0, 5, 25, or 100 mg/kg/day by gavage on gestation days 6-15. Developmental NOEL = NOAEL = 5 mg/kg/day [fused sternebrae (#1 and #2)], a **"possible adverse effect"** for this metabolite. Ossification delays were common at 100 mg/kg/day. Maternal NOEL = 5 mg/kg/day (minor decrements in body weight and food consumption). **Acceptable** as an ancillary study. (H. Green and C. Aldous, 10/3/96).

220-226 128822 Marty, J. H., "Developmental toxicity (teratogenicity) study in rats with G-30033 Technical (oral administration)," Ciba-Geigy Limited, Reproduction Toxicology, Stein, Switzerland, Report # 901265, 1 June 1992. G-30033 technical, 97.5% pure, was administered by gavage to Tif: RAI f (SPF) rats, 24 mated females per group, at 0, 5, 25, or 100 mg/kg/day on gestation days 6-15. Maternal NOEL = 5 mg/kg/day (slightly reduced food consumption at 25 mg/kg/day). Maternal effects at 100 mg/kg/day included more pronounced reduction in food consumption, statistically significant b.w. decrement, and hunched posture in 1 dam. Developmental NOEL = 25 mg/kg/day (sternebrae #1 and #2 fused). **Acceptable** as an ancillary study, with a "possible adverse effect" (fused sternebrae). H. Green, and C. Aldous, 10/3/96.

220-227 128823 Hummel, H. et al., "Diaminochlorotriazine, A teratology (Segment II) study in rats," Ciba-Geigy Corporation, Summit, NJ, 8/15/89, Report No. 89043. Diaminochlorotriazine (DACT) with at least 98.1% purity. Crl:COBS CD (SD)BR rats, 26 per group, received 0, 2.5, 25.0, 75.0, or 150.0 mg/kg/day by gavage on gestation days 6 through 15. Maternal NOEL = 25 mg/kg/day (maternal food consumption and body weight gain decrements). Developmental NOEL = 2.5 mg/kg/day (ossification delays in parietal, interparietal, and hyoid bones at ≥ 25 mg/kg/day). Changes at 75 to 150 mg/kg/day included dose-related decrements in fetal body weights, and ossification delays in the skull, hindpaw, and ribs. At 150 mg/kg/day there was also an increase in resorptions. **No adverse effects** (considering that developmental effects at all dose levels below 150 mg/kg/day appeared to be delays in development, without evidence of permanent changes). **Acceptable**. (H. Green and C. Aldous, July 6, 1995).

220-230 128905 Hummel, H. et al., [Analytical confirmation of identity of diaminochlorotriazine]. Relates to 220-227:128823, above. Test article is consistent with diaminochlorotriazine by MS, IR, and NMR. Aldous, 7/5/95.

Reproductive Toxicity Mechanisms (Atrazine-Related Compounds):

220-218 128788 Eldridge, J. C., "Interactions of simazine, a chlorotriazine herbicide, with the estrogen receptor system of rat uterus," Bowman Gray School of Medicine of Wake Forest University, 4/26/91. Several in vitro studies, utilizing pooled uterine cytosol, investigated competition between simazine and 3H-estradiol for specific estrogen receptor interactions. Simazine proved to be a weak competitive inhibitor of estrogen. Investigators determined that at simazine loading levels that might occur during chronic studies (e.g. 100 mg/kg b.w.), it is possible that simazine could compete with or delay binding of biologically significant amounts of estrogens with receptors. Aldous, 10/4/96 (no worksheet).

Mutagenicity (Atrazine-Related Compounds):

Test Type 842

** 220-408 139098 "Diaminochlorotriazine: Gene mutations test: Salmonella/mammalian-microsome mutagenicity test" (E. Deparade, Ciba-Geigy Limited, Switzerland, Lab study number 871372, 11/10/87) Diaminochlorotriazine (G28273) technical, 97% purity, was tested with Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 with and without activation with Aroclor 1254-induced rat liver S9. Concentrations were 0 (DMSO), 20, 78, 313, 1250, or 5000 mg/plate, triplicate plates per concentration, two trials. A precipitate formed at 5000 mg/plate. Positive controls with and without activation were functional. No evidence of an increase in reversion frequency with treatment was reported under the test conditions. **No adverse effect. Acceptable.** (Gee, 8/26/96)

** 220-408 139100 "Hydroxyatrazine: Gene mutations test: Salmonella/mammalian-microsome mutagenicity test" (E. Deparade, Ciba-Geigy Limited, Switzerland, test no. 871376, 2/15/88) Hydroxyatrazine [G34048] technical, 99% purity, was tested for mutagenicity with Salmonella strains TA98, TA100, TA1535 and TA1537, with and without rat liver activation with triplicate plates and two trials. Concentrations tested were 0 (DMSO), 20, 78, 313, 1250 and 5000 µg/0.1 ml/plate with 48 hour incubation. No increase in reversion rate was reported under the test conditions. **No adverse effect. Acceptable.** (Gee, 8/22/96)

** 220-233 128908, "G-30033 Technical, Gene Mutations Test, Salmonella and Escherichia/Liver-Microsome Test," (Eckhard Deparade, Ciba-Geigy Limited, Basel, Switzerland, Report # 891236, 18 December 1989). G-30033 technical (deethylatrazine), 99.3% purity. Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 and Escherichia coli strain WP2uvrA were exposed for 48 hours in the presence and absence of rat liver microsomal activation to concentrations of 0 (DMSO), 313, 625, 1250, 2500, and 5000 mg/0.1 ml/plate. **No increase in reversion frequency indicated. Acceptable.** (H. Green and Gee, 8/29/96)

** 220-232 128907, "G-28279 Technical, Gene Mutations Test, Salmonella and Escherichia/Liver-Microsome Test," (Eckhard Deparade, Toxicology II, Ciba-Geigy Limited, Basel, Switzerland, Report # 891243, 18 January 1990). The test article was G-28279 technical

[deisopropylatrazine], 97.4% purity. Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 and Escherichia coli strain WP2uvrA were exposed for 48 hours in the presence and absence of rat liver microsomal activation at 0 (DMSO), 313, 625, 1250, 2500, and 5000 mg/0.1 ml/plate, triplicate plates, two trials. **No increase in reversion frequency was indicated** under test conditions. **Acceptable.** (H. Green and Gee, 8/29/96).

Test Type 843

** 220-408 139108 “Hydroxyatrazine: Structural Chromosomal aberration test; micronucleus test, mouse,” (Ceresa, Ciba-Geigy Limited, Switzerland, Lab Study No. 871373, 8/31/88) Hydroxyatrazine [G34048] technical, 99% purity, was tested with Tif: MAGF, SPF mice of both sexes for the induction of micronuclei in polychromatic erythrocytes of the bone marrow. The study was conducted in two parts. Part 1: 24 mice per sex were given a single dose of 0 (0.5% carboxymethylcellulose) or 5000 mg/kg hydroxyatrazine. Eight per sex were sacrificed at 16, 24 or 48 hours after dosing and the bone marrow harvested for micronuclei analysis. Cyclophosphamide (64 mg/kg) was used as the positive control. Part 2: 8/sex were given 0, 1250, 2500 or 5000 mg/kg in a single dose with sacrifice after 24 hours. Slides from 5 per sex per group of eight were evaluated for the number of micronuclei and the ratio of PCE/NCE per animal. There was no increase in the formation of micronuclei with exposure to hydroxyatrazine. **No adverse effect** under the test conditions. **Acceptable.** (Gee, 8/23/96)

** 220-235 128910, “G-30033 Technical, Structural Chromosomal Aberration Test, Micronucleus Test, Mouse,” (B. Ogorek, Genetic Toxicology, Ciba-Geigy Limited, Basel, Switzerland, Report # 901309, 25 March 1991). The test article was G-30033 technical [deethylatrazine] with 99.3% purity. In the first stage, 24 Tif: MAGf (SpF) mice per sex per group received 0 (0.5% carboxymethylcellulose) or 480 mg/kg by gavage. Five (5) per sex per group were sacrificed at 16, 24, or 48 hours after treatment. In a second part, 8 per sex per group received 0, 120, 240, and 480 mg/kg. Bone marrow from 5 per sex per group was sampled 24 hours after treatment. **No increase in micronucleated polychromatic erythrocytes was reported.** **Acceptable.** (H. Green and Gee, 8/29/96).

** 220-234 128909, “G-28279 Technical, Structural Chromosomal Aberration Test, Micronucleus Test, Mouse,” (B. Ogorek, Genetic Toxicology, Ciba-Geigy Limited, Basel, Switzerland, Report # 901307, 23 February 1991). The test article was G-28279 technical [deisopropylatrazine], 97.4% purity. In part one, Tif: MAGf (SpF) mice received 0 (0.5% carboxymethylcellulose) or 480 mg/kg by gavage, then marrow samples from 5/sex/treatment were evaluated 16, 24, or 48 hours after treatment. In part two, mice received 0, 120, 240, or 480 mg/kg. Bone marrow samples from 5 per sex per group were harvested 24 hours after treatment. One thousand (1000) PCE's were scored per animal. **No increase in micronucleated polychromatic erythrocytes was found.** **Acceptable.** (H. Green and Gee, 8/28/96).

Test Type 844

220-408 139099 “Diaminochlorotriazine: Tests for other genotoxic effects: Autoradiographic DNA repair test on human fibroblasts” (A. Meyer, Ciba-Geigy, Switzerland, lab number 871371, 11/20/87). Diaminochlorotriazine technical [G28273], 97% purity, was tested with human

fibroblast cell line CRL 1521 (ATCC), passages 8, 13 and 14 without activation only. Concentrations tested were 0 (medium, DMSO), 5.56, 16.67, 50, 150, 300 or 600 µg/ml, five hours, triplicate coverslips, two trials. The positive control was 4-nitroquinoline-N-oxide. Concentrations were based on a preliminary cytotoxicity test with concentrations to 1000 µg/ml with evaluation of percent viable by dye exclusion and adhesion/morphological evaluation. Unscheduled DNA synthesis was determined by autoradiography following incorporation of ³H-thymidine into nuclear DNA. Cell-free areas of the slides were used for background. Fifty cells from each of three slides per concentration were scored. **No adverse effect of unscheduled DNA synthesis induction** under the test conditions reported. **Unacceptable, not upgradeable** (no activation included, inadequate details regarding minimization of S-phase DNA synthesis, no cytoplasmic counts). (Gee, 8/26/96)

220-408 139103 "Hydroxyatrazine: Tests for other genotoxic effects; Autoradiographic DNA repair test on human fibroblasts" (A. Meyer, Ciba-Geigy Limited, Switzerland, lab no. 871375, 1/11/88) Hydroxyatrazine [G34048] technical, purity of 96-99%, was tested with the human fibroblast cell line CRL 1521 for the induction of unscheduled DNA synthesis by autoradiography. Cells were exposed for five hours without activation only to concentrations of 0 (DMSO), 13.89, 41.67, 125, 375, 750 or 1500 µg/ml in multiplates containing coverslips. Four replicates per concentration were exposed per trial (two trials) with fifty cells from three coverslips scored for each replicate. Cell-free areas were used as background. No measures were taken to minimize S-phase DNA synthesis. No evidence for the induction of unscheduled DNA synthesis under the test conditions was reported. **No adverse effect. Unacceptable** (no activation included). Not upgradeable. (Gee, 8/22/96)

** 220-408 139105 "Hydroxyatrazine: Test for other genotoxic effects: autoradiographic DNA-repair test on rat hepatocytes" (Th. Hertner, Ciba-Geigy Limited, Switzerland, lab. no. 871374, 1/22/88) Hydroxyatrazine [G34048] technical, 96-99% purity, was tested with male rat hepatocytes for the induction of unscheduled DNA synthesis. Primary hepatocytes were exposed on coverslips for 16-18 hours in two trials. First trial: 0 (DMSO, medium), 13.89, 41.67, 125, 375, 750 or 1500 µg/ml. Second trial: 0, 3.125, 6.25, 12.5, 25, 50, 100, 200, 300, 500 or 1500 µg/ml. Precipitates were visible at concentrations of 12.5 µg/ml and higher. Concentration selection was based on a preliminary cytotoxicity test. Unscheduled DNA synthesis was assayed by autoradiography following incubation with ³H-thymidine. Fifty nuclei/slide for each of three slides were scored per concentration. No consistent evidence was reported for the induction of unscheduled DNA synthesis with hydroxyatrazine under the test conditions. **No adverse effect. Acceptable.** (Gee, 8/26/96)

** 220-236 128911, "G-28279 Technical, Tests for Other Genotoxic Effects, Autoradiographic DNA Repair Test on Rat Hepatocytes," (D. Geleick, Genetic Toxicology, Ciba-Geigy Limited, Basel, Switzerland, Report # 901308, 12 April 1991). The test article was G-28279 technical, 97.4% purity. Hepatocytes from adult male Tif.RAIf (SPF) rats were treated in quadruplicate at 0 (DMSO), 7.4, 22.2, 66.6, 200, 400, and 800 mg/ml for 16 to 18 hours. Unscheduled DNA synthesis was determined by incorporation of ³H-thymidine and quantitated by autoradiography. **No induction of unscheduled DNA synthesis was indicated** under test conditions. **Acceptable.** (H. Green and Gee, 8/28/96).

** 220-237 128912, "G-30033 Technical, Tests for Other Genotoxic Effects, Autoradiographic DNA Repair Test on Rat Hepatocytes," (D. Geleick, Genetic Toxicology, Ciba-Geigy Limited, Basel, Switzerland, Report # 901310, 26 April 1991). The test article was G-30033 technical [deethylatrazine] with 99.3% purity. Primary hepatocytes from male Tif: RAIf (SPF) rats were

exposed in quadruplicate to test material concentrations of 0 (DMSO), 9.25, 27.7, 83.3, 250, 500, and 1000 mg/ml for 16 to 18 hours. Unscheduled DNA synthesis analyzed by autoradiography. **No increase in unscheduled DNA synthesis** under test conditions. **Acceptable.** (H. Green and Gee, 8/29/96).

Studies of Atrazine Together with Other Active Ingredients and/or Fertilizers in Ground Water Contamination Studies:

220-364 137857 Johnson, E. M., "The effects of representative groundwater pesticides on reproduction and in utero development of experimental animals," (not a study, but a brief critique of 2 developmental toxicity and two reproduction studies employing such mixtures), 6/22/93. Components that commonly contaminate Iowa or California groundwaters, respectively, were tested at up to 100 times the median observed levels in respective areas in "CR" rats (developmental studies) and in CD-1 mice (continuous breeding reproduction studies). The only noteworthy finding was an 11% reduction in seminal vesicle weights in the mouse reproduction study employing the 100-fold California groundwater mixture (without any associated histopathology). No DPR worksheet is relevant for this brief critique. Aldous, 7/25/95.

A number of new studies, mainly on metabolites of atrazine, have been received. Metabolite studies may be evaluated in the future if warranted by ubiquity in the environment, or evidence of toxicity concern. Abstracts of studies have been examined in any case. J. Kishiyama and C. Aldous, 4/20/94.

CROSS INDEX OF CHEMICALS (IF CHARACTERIZED) AND CODE NUMBERS

[from 220-437:146019, pp. 14-15 (Ciba Study #174-91)]

CODE NAME OR DESCRIPTION OF TEST ARTICLE WHERE IDENTIFIED

G-28273 Diaminochlorotriazine

i.e. 2,4-diamino-6-chloro-s-triazine

G-34048 Hydroxyatrazine

i.e. 2-ethylamino-4-hydroxy-6-isopropylamino-s-triazine

G-28279 deisopropylatrazine

i.e. 2-amino-4-chloro-6-ethylamino-s-triazine

G-30033 deethylatrazine

i.e. 2-amino-4-chloro-6-isopropylamino-s-triazine

GS-17794 deethylhydroxyatrazine

i.e. 2-amino-4-hydroxy-6-isopropylamino-s-triazine

GS-17792 deisopropylhydroxyatrazine

i.e. 2-amino-4-hydroxy-6-ethylamino-s-triazine

GS-17791 diaminohydroxyatrazine

i.e. 2,4-diamino-6-hydroxy-s-triazine

- end of Summary of Toxicology Data 1-liners -

NOTE: From a DPR Library search of 11/15/07, the following records were not considered relevant for this summary:

220-0554 178403, 178404, 178405, 178406, 178407, 178408, and 178409. Atrazine: Various Journal Articles and Studies for Risk Assessment (106 pages). Multiple Sources: Company and Cooperator Field Reports (Cooperator May Be University, Farmer, Farm Advisors, etc.). This volume was received at DPR on 11/30/2000. This volume contains 8 small reports, primarily on exposure and risk assessment subjects. This volume was routed directly to MTB Risk Assessment Group, and assigned to D. Gammon. Records of interest to Dr. Gammon were examined on 8/24/01. None of these studies require MTB Data Review Group evaluation at this time. Aldous, 11/16/07.